# HISTOPATHOLOGICAL ANALYSIS AND EXPRESSION OF p16 IN SQUAMOUS CELL CARCINOMA OF UPPER-AERODIGESTIVE TRACT

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**CHENNAI, TAMILNADU**

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This is to certify that the dissertation entitled **"HISTOPATHOLOGICAL ANALYSIS AND EXPRESSION OF p16 IN SQUAMOUS CELL CARCINOMA OF UPPER-AERODIGESTIVE TRACT"** submitted by **Dr.M.Meenambigai** to the Faculty of Pathology, The Tamilnadu **Dr.M.G.R. Medical University, Chennai** in partial fulfilment of the requirement for the reward of **M.D.Degree in Pathology** is a bonafide work carried out by her during the period 2016-2018.

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# DECLARATION BY CANDIDATE

I, **Dr.M.Meenambigai** solemnly declare that the dissertation titled **'HISTOPATHOLOGICAL ANALYSIS AND EXPRESSION OF p16 IN SQUAMOUS CELL CARCINOMA OF UPPER-AERODIGESTIVE TRACT'** is a bonafide work done by me at Department of Pathology, Madurai Medical College &Government Rajaji Hospital, Madurai during the period from july 2016 to july 2018.

I also declare that this bonafide work or a part of this work was not submitted by me or any other for any reward, degree and diploma to any university, board either in India or abroad.

The dissertation is submitted to The Tamilnadu **Dr.M.G.R. Medical University**, towards partial fulfillment of requirement for the reward of **M.D. Degree in PATHOLOGY.**

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# INTRODUCTION

The upper-aerodigestive tract (UADT) comprises of nasal cavity, nasopharynx, oral cavity, oropharynx, larynx, trachea. Upper-aerodigestive tract tumors forms 4% of all malignancies. Squamous cell carcinoma(SCC) is the common tumor, with male : female ratio of 3:1. Common risk factors are smoking, tobacco and alcohol consumption. Human Papilloma Virus (HPV) is a newly diagnosed risk factor and it is mostly seen in absence of other risk factors. HPV associated SCCs of upperaerodigestive tract is seen in a unique population of patients, i.e they are younger in age compared to the conventional SCC and these tumors have characteristic histopathological feature : They are non – keratinizing basaloid appearing tumors with necrosis and palisading seen in areas. These also have better response to treatment than the conventional SCC.

The Human Papilloma virus contributes to carcinogenesis by expressing E6 and E7 oncoproteins, which binds to p53 and Retinoblastoma gene. These proteins are important for normal cell cycle regulation and when these proteins are bound by the viral oncoproteins, they get disrupted causing the cells to proliferate with increased expression of the tumour suppressor protein p16.

More than 86% of HPV associated tumours show increased expression of p16.

Hence, studies are needed to understand the biology of HPV related upper aerodigestive SCC in our Indian population is a must, as we have higher incidence of oral cancers compared to the west .

Present study is done to evaluate the association of HPV in upper aerodigestive tract squamous cell carcinoma using p16 immunostaining .

## AIMS AND OBJECTIVES

- To study the frequency of occurrence of squamous cell carcinoma in specimens received in the Department of pathology, Madurai Medical College, Madurai.
- To study age, sex and site related incidence of squamous cell carcinoma.
- To study the histopathological features of squamous cell carcinoma
- To classify squamous cell carcinoma based on World Health Orgaisation classification.
- To correlate the histological variants of squamous cell carcinoma with Human papilloma virus by using p16 expression as a surrogate marker.

# Evolution of squamous cell carcinoma of upper aerodigestive tract

**Squamous** →literally ”means” a layer of epithelium that consists of thin flattened cells, like scales of fish and they are found in the tissue that forms the surface. For example: skin and the epithelial lining of the hollow organs of the body.

The carcinoma arising from this epithelium is called as epidermoid / squamous cell carcinoma.

Classification of squamous cell carcinoma and its relation with patient outcome is studied for many years. **It was started in 1920** by “**Broder**”. He first attempted histological grading system to determine the prognosis of squamous cell carcinoma of lip. He graded tumors into 4 types taking into account the degree of keratinization seen in the tumor cells. [1-3]

Well differentiated – 75% - 100% of tumor cells show keratinization.

Moderately differentiated – 50% - 75% of tumor cells show keratinization.

Poorly differentiated – 25% - 50% of tumor cells show keratinization.

Anaplastic tumor – 0% - 25% of cells show keratinization.

This system showed high intra and inter observer variation and also this histological grading was found to have a poor correlation with the patient outcome.

**In 1973, Jaccobson et al** saw pit falls in Broder's classification and suggested a mutlifactorial grading system which includes : Degree of differentiation, mitosis, nuclear pleomorphism, stage of invasion, mode of invasion, vascular invasion and lymphoplasmacytic infiltrate.[4]This system had more number of parameters.

**In 1987, Anneroth** modified the jaccobson's multifactorial grading system and included; Nuclear pleomorphism, degree of keratinization, mitosis, pattern and stage of invasion and lymphoplasmacytic infiltrate in the tumor margins.[5]Each parameters scored from 1-4 and based on the total score the tumor was graded as:

Grade I – Score of 6 to 12

Grade II – Score of 13 to 18

Grade III – Score of 19 to 24.

However some other histologically important prognostic points were not taken in this classification, like perineural tumor invasion, lymphatic and vascular invasion and tumor thickness.

**Lima dantas & Dilana duarete** found there was no correlation of Anneroth's classification of scoring SCC with the prognosis of the patient.

**In 1992, Bryne's et al** proposed a grading system based on deeply invasive tumor margins. In this system, two things of Anneroth grading system was removed (Mitosis and stage of invasion) and remaning parameters are seen in the deepest tumor margin. They were scored and based on score they were graded as :

Grade I – Score of 4 to 8

Grade II – Score of 9 to 12

Grade III – Score of 13 to 16.

**Yazdi et al,** showed that there was significant statistical correlation between the Bryne's scoring of SCC & lymph node metastasis which in turn determine the prognosis of the patient. (6)

**In 1940**, **Pierre denoix** first proposed **TNM** staging for 23 sites in body, which was later adapted by **UICC**. It is a classification system to find the anatomical extent of the tumor, which determines the patient outcome. This system was also followed by **AJCC** and **FIGO**. In the year **1987** both **AJCC** and **UICC** classification was combined and a

single TNM classification was formed. After that it had gone for continuous revision and the last edition was released in 2017.

In addition attempts were made to obtain data for prognostic features by other methods which includes molecular markers like p53, HER 2 and p16 overexpression.

## DEVELOPMENT AND ANATOMY OF UPPER AERODIGESTIVE TRACT

**Embryogenesis:**

The important part in development of head and neck region is formation of pharyngeal arches. They develop around 4$^{th}$ week. It consists of mesoderm separated by deep clefts. This lead to development of pouches. These pouches donot communicate with clefts. Paraxial and lateral plate mesoderm contribute to pharyngeal arch mesoderm. The arches are lined externally by ectoderm, internally by endoderm. Mesoderm gives rise to muscles of face and neck. Few neural crest cells also migrate inside the mesoderm and forms the muscles. And these arches also have its own nerves and artery.

**First Pharyngeal Arch:**

It give rise to maxillary, mandibular processes, muscles of mastication, anterior belly of digastrics, myohyoid, tensor palatine, tensor tympani, trigeminal nerve[maxillary & mandibular] and bones of face and skull, malleus, incus, spenomandibular ligament and anterior ligament of malleus.

**Second Pharyngeal Arch:**

It give rise to muscles of facial expression, stapedius, stylohyoid, posterior belly of digastric, facial nerves, portion of hyoid bone, stapes, styloid process and stylohyoid ligament.

**Third Pharyngeal Arch:**

It gives rise to stylopharyngeous muscle, glossopharyngeal nerve and remaining part of hyoid bone.

**Fourth Pharyngeal Arch:**

It give rise to constrictors of pharynx, cricothyroid, levator palatine, superior laryngeal nerve and cartilages of larynx.

**Sixth Pharyngeal Arch:**

Intrinsic muscles of larynx, recuurent laryngeal nerve and cartilages of larynx.

**Pharyngeal Pouches:**

There are 4 pairs of pharyngeal pouches. Derivatives of these pouches are:

1st pharyngeal pouch: Middle ear cavity and eushtachian tube

2nd pharyngeal pouch: Palatine tonsils with tonsillar fossa

3rd pharyngeal pouch: Thymus & inferior parathyroid glands

4thpharyngeal pouch: Ultimobranchial bodies and superior parathyroid gland

**Pharyngeal Clefts:**

Of the 5 clefts, only the first cleft contribute to the development of embryo. They form the external auditory meatus with the ear drum and merges with the epicardial ridge seen in the lower part of neck. (7)

**Development of Nasal cavity:**

By end of 4th week, centre of face has stomodeum surrounded by first pharyngeal arch and stomodeum is covered by oro-pharyngeal membrane.

By end of 6th week, 5 mesenchymal prominences are recognized. They are [from above to below]

*Frontonasal prominence

*Maxillary prominence

*Mandibular prominence

Around 5th week there is deepening of nasal placode to form the nasal pits. These pits deepens into the underlying mesenchyme with

simultaneous growth of the nasal prominences. The oropharyngeal membrane seperates the pit from the primitive oral cavity.

Later there is rupture of the membrane, so the nasal cavity and oral cavity communicate through primitive choanae. This choanae lies on either side of the midline and behind the primary palate. After the development of secondary palate, the choanae lies at junction of pharynx and nasal cavities. This will be completed around 9$^{th}$week.

Tip of nose, crest and philtrum of upper lip is formed from medial nasal prominence. Ala of nose from fusion of lateral nasal with maxillary prominence.

Nasal septum develops from the frontal prominence and fuses with the intermaxillary segment below. Inter maxillary segment is developed from fusion of medial nasal prominence with maxillary prominence. It has 3 parts: Labial part --> philtrum of upper lip.

Upper jaw part --> carries 4 incisor teeth

Palatal part --> triangular primitive palate.

Before fusion of the maxillary and lateral nasal prominence there is a groove called the nasolacrimal groove. The epithelium from the ectoderm of this groove later forms the naso-lacrimal duct. The turbinates

develop as elevation from lateral nasal wall. The paranasal sinuses develop as diverticula from the lateral wall and into the maxilla. (7)

**Development of Oral Cavity:**

After development of the primary palate, secondary palate develops from palatine process [Extension from maxillary prominence] by 6th week. It lie obliquely downward on either side with the tongue below. In 7th week it ascend and lie parallel to the tongue and fuse in midline. At junction of the primary and secondary palate fusion in the midline incisive foramen is formed.

Anterior 2/3rd of tongue develops from tuberculum impar, two lateral and one medial lingual swellings[first pharyngeal arch]. This part is innervated by mandibular branch of trigeminal nerve.

Posterior 1/3rd develops from the medial eminence called the hypobranchial eminence[2nd, 3rd and 4th arches] and innervated by glossopharyngeal nerve.

Epiglottis develops from one medial eminence[4th arch] and innervated by superior laryngeal nerve.

Special sensory innervations to anterior 2/3rd supplied by chorda tympani of facial nerve and posterior 1/3rd by glossopharyngeal nerve.

Muscles of tongue develops from the occipital somites and innervated by hypoglossal nerve.

Soft palate formed by the tendons of palati muscles[Tensor veli palate, Levator veli palate, Palatopharyngeus, Palatoglossus and uveal muscles] [8-9]

**Anatomy of Nasal Cavity:**

It is bounded by a roof, floor, medial and lateral walls. Medial wall by nasal septum. Lateral wall by perpendicular plate of palatine bone, lacrimal bone, maxilla, pterygoid plate of sphenoid bone, ethmoid labrynith, superior, middle & inferior concha. Roof by cribriform plate of ethmoid. Floor by hard palate, palatine process of maxilla. Anterior apertures are called– Nares. Posterior apertures are called Choanae, which open into nasopharynx.

**Anatomy of Nasopharynx:**

It lies behind choanae, above the soft palate is bounded by: Roof – Base of the skull [ Body of sphenoid & part of occipital bone], Floor – soft palate(anteriorly) with defect posteriorlyforming naso-pharyngeal isthmus, Lateral wall –Mucosal folds and covering of the pharyngeal opening of eushtachian tube, Anterior – choanae of nasal cavity, Posterior – Arch of atlas vertebra with muscles and fascia. Lymphatics drain into the upper deep cervical nodes.

**Anatomy of Oral Cavity:**

It extends from lips to pro-pharyngeal isthmus. Lips form the anterior boundary, buccal mucosa lines the inner surface of the cheeks, gums(gingivae) surround the teeth and cover the alveolar ridges, retromolar trigone form a triangular area of mucosa covering anterior surface of ramus of mandible, hard palate forms the roof of oral cavity. Anterior 2/3$^{rd}$ tongue is present inside oral cavity and is mobile. It has tip, two lateral borders, dorsum and undersurface. Floor of mouth is the area between the gingivae and undersurface of tongue.

**Lymphatics**

Upper lip drain into: Preauricular, infraparotid and submandibular nodes.

Lower lip, alveolar ridges and floor of mouth drain into submental and submandibular nodes.

Hard palate drain into upper deep cervical and lateral retropharyngeal nodes.

Tongue drain into submental, submandibular and deep cervical nodes. [8]

**Anatomy of Oro-Pharynx:**

Extends from hard palate to hyoid bone.

**Anterior wall:** Through oropharyngeal isthmus it communicate with oral cavity. Posterior 1/3$^{rd}$ of tongue( upper part) with lingual tonsils, vallecula(depressions in anterior surface of epiglottis) forms the wall.

**Lateral wall:** Palatoglossal arch(anterior pillar), palatopharyngeal arch(posterior pillar). Between these arches lies the tonsillar fossa with palatine tonsils.

**Posterior wall:** Is formed by retropharyngeal space. Lymphatics drain into the jugulo-digastric node.

**Anatomy of Hypopharynx**:

It is the lower part of pharynx and lies behind and on sides of larynx. Extends from hyoid bone to the cricoid cartilage. It is clinically divided into: Pyrifrom fossa, posterior pharyngeal wall and post- cricoid region.

**Pyriform fossa:** It lies on either side of larynx and is bounded laterally by thyrohyoid membrane, thyroid cartilage. Medially by aryepiglottic fold, cricoid and arytenoid cartilages. Lymphatics drain into upper jugular chain.

**Post -cricoid region:** Forms part of anterior wall of hypopharynx.

Lymphatics drain into parapharyngeal nodes.

**Posterior pharyngeal wall:** Extends from hyoid bone to cricio-arytenoid joint. Lymphatics drain into parapharyngeal nodes. [9]

**Anatomy of Larynx**:

It is divided into supra glottic, glottic and sub glottic areas.

**Supra Glottic Region**:

It extends from tip of the epiglottis above to the ventricle below. It consists of the epiglottis, aryepiglottic folds, arytenoid cartilages, false cords and the ventricle. It is further divided into suprahyoid and infrahyoid areas. Lymphatics drain from here through the thyrohyoid membrane and into the subdigastric and superior jugular nodes.

**Glottic region:**

It consists of true vocal cords, anterior and posterior commissures. There are no lymphatics for the true vocal cords. The anterior commissure (broyle's ligament) has a band of fibrous tissue. The lymphatics from here drain along the lymphatics of thyroid cartilage. The lower border of glottis region lies one cm below the apex of the ventricle.

**Subglottic region:**

It lies below the glottis to the lower border of the cricoid cartilage. The lymphatics from this region drain into the paratracheal node, deep cervical node, and prelaryngeal node. Intra laryngeal spatial subdivision

is done, as in most of SCC of larynx the surgical procedures are done based on this spatial division only. The different spaces are : Supraglottic space, pre- epiglottic space, para glottic space, reinkes space and sub glottic space. (10)

## HISTOLOGY OF UPPERAERODIGESTIVE TRACT

**Lip** on its external surface is lined by stratified squamous epithelium of skin with hair, which passes through a transition zone called the vermilion border and becomes continuous with the epithelium of oral cavity. Vermilion border is free of hair and adnexal glands. This layer is highly vascular and has more sensory innervations. Submucosal layer in inner surface of lip has many minor salivary glands. **Oral cavity** is lined by stratified squamous epithelium. Keratinisation is seen only in areas of friction. Lamina propria forms the supporting framework with dense collagenous tissue. In mobile parts of the oral cavity like soft palate and base of tongue, lamina propria is tightly adherent to the submucosa and also has many serous and mucous minor salivary glands. **Tongue** is a muscular organ. The V shaped sulcus called the sulcus terminalis separates the anterior 2/3$^{rd}$ from posterior 1/3$^{rd}$ of tongue. Mucosa of anterior 2/3$^{rd}$ is formed into papillae. They are of three types. Predominant form is filliform papillae seen macroscopically as short bristles. Globular fungiform papillae are seen scattered between the

filliform papillae. The third type circumvallate papillae is seen in front of sulcus terminalis, it contains numerous taste buds. The posterior 1/3rd of tongue is covered by smooth stratified squamous epithelium. Underlying dense lymphoid aggregates form the lymphoid follicles and is called lingual tonsil. Lingual tonsil, palatine tonsils, pharyngeal and tubal tonsils form the waldeyer ring. The stratified squamous epithelium lining the tonsils form deep clefts called tonsillar crypts. Tubal tonsils also called the adenoids are the only tonsils lined by pseudostratified ciliated columnar epithelium. Parenchyma of all tonsils have lymphoid follicles forming the germinal centre and surrounding paracortical area. (11)

**Nasal cavity** and paranasal sinuses are lined by respiratory epithelium i.e. pseudostratified ciliated columnar epithelium with mucin secreting goblet cells. Beneath the basement membrane of the epithelium, the lamina propria contains the serous and mucous glands. These secretions help in trapping smaller particulate that has passed through nares. Main function of respiratory epithelium is to maintain the temperature and humidity of the inhaled air. The roof of nasal cavity is lined by olfactory mucosa, which contains receptors for smell.

**Nasopharynx** is lined by respiratory epithelium. Squamous lining seen in some areas in smokers and in advanced age. An important component in the lamina propria of nasopharynx is dense lymphoid

aggregates called the adenoids. These are prominent is children and young adults.

**Laryngeal** aspect of the epiglottis are covered by nonkeratinized stratified squamous epithelium. It merges with the respiratory epithelial lining the false cords and ventricle. Beneath the epithelium sero-mucinous glands are seen. Few scattered small granular cells with neurosecretory granules are seen, which can be demonstrated by immunohistochemistry. They belong to neuroendocrine system. The true cords is made of vocalis muscle and ligament is lined by stratified squamous epithelium.

**Hypopharynx** is lined by non- keratinizing stratified squamous cell. [12 – 14]

## EPIDEMIOLOGY OF CANCER IN UADT

Head and neck cancer ranks 3rd in overall cancers in developing countries and ranks 5th globally. And squamous cell carcinoma is the most common carcinoma of upper aerodigestive tract, constituting > 90% of all the UADT carcinomas. It has higher incidence in men than women, with mean age group affected range from 5th to 7th decade. The epidemiological data for different sites in UADT are as follows: Incidence of lip cancer is 0.9% in Asia and uncommon in women. Higher incidence of world wide cancers of oral cavity and pharynx is from

southeast Asia, with equal sex incidence in India. There is predominance of oral cancer in male sex in other parts of world. Cancer of post-cricoid region is common in female population both in Asian and European population and 30% - 70% of these cancers are found in association with Plummer Vinson syndrome. Also called as Paterson Kelly syndrome / Sideropenic dysphagia. If patients have this syndrome, 3% -7% develop post cricoid cancer. Cancer of nose and paranasal sinuses are infrequent world wide, but common in Japan. They have incidence of 2.2 – 2.6 per 1 lakh population in males, 1.2 – 1.4 per/lakh population in females. Naso-pharyngeal cancers are higher in southern china and Africa, with male preponderance. Age group varies with 45-55yr in high endemic areas and more in adolescents in areas with low incidence. [15] Laryngeal cancer occurs in 6-7$^{th}$ decade with M:F ratio of 5:1 and commonly affect the black population than the whites. It has higher incidence in southern Europe.

## RISK FACTORS

### Behaviour Risk Factors:

Tobacco and alcohol are the most important risk factors in development of upper- aerodigestive cancer.

Betel quid, areca nut, lime, leaf of betel vine and tobacco has higher incidence of buccal cavity, alveolar and tongue cancers.

Smoking is associated more with laryngeal and hypopharyngeal cancer. Alcohol consumption causes higher chance of floor of mouth, hypopharynx and supraglottis cancers.

**Infections:**

Viruses like human papilloma virus, Epstein barr virus are identified risk factors.

Epstein barr virus( EBV) is associated with nasopharyngeal carcinomas, including keratinizing squamous cell carcinoma and undifferentiated carcinoma. It is positive mostly in Asian population. EBV binds with the B cells and epithelial cells through CD21 receptor and proliferates inside the cells by activation of NF-kB pathway. This increases the expression of many factors like, VEGF, matrix metallo protinases etc that promote in development of cancer. Viral oncogenes LMP(Latent membrane protein-1) and EBNA2 play the role in carcinogenesis of EBV. [16]

Human papilloma virus(HPV) is recently identified as risk factor of non-keratinizing carcinoma, especially in areas like, tonsillar fossa, posterior third of tongue and in sinonasal tract. Among various types, type16 is commonly involved in carcinoma of upper-aerodigestive tract. HPV block the function of p53 and RB, activate cyclins, cyclin dependent kinases and increases the proliferative activity of the epithelial cells.

There by they form pre-malignant lesion and carcinoma. E6 and E7 viral genes are responsible for the oncogenic potential of HPV. [17–18]

**Occupational Exposure:**

Exposure to softwood dust, nickel, mustard gas are associated with squamous cell carcinoma. Other factors like exposure to hard wood, chrome pigment, leatherdust and thorotrast are associated with adenocarcinoma especially in sino-nasal regions. [19- 22]

**Food Habits:**

In certain areas like, southern china, artic region and Eskimos consumption of salt – cured fish is associated with increased incidence of nasopharyngeal cancers. Excessive consumption of a non-alcoholic drink called mate drink, which is prepared from leaves of the herb plant Ilex paraguariensis (Yebra mate) is also associated with carcinoma of oral, oro-pharyngeal and esophageal cancers. It was initially found in latin America. [23-24]

## SYNDROMES ASSOCIATED WITH CANCERS OF UPPER AERO-DIGESTIVE TRACT

Few inherited syndromes are associated with carcinomas of upper-aerodigestive tract. They are: Bloom's, xeroderma pigmentosum, fanconi anemia, li-fraumeni syndrome.

Fanconi anemia is an autosomal recessive disorder, associated with increased risk of malignancies of head and neck region. Common sites are base of tongue, pyriform fossa, post-cricoid region and gingiva. They also have higher chance of association with human papilloma virus related malignancies.

Xeroderma pigmentosum, were there is defect in DNA excision repair system is an autosomal recessive condition. On exposure to ultra-violet light DNA damage occurs. And they have higher incidence of carcinoma of anterior 2/3$^{rd}$ of tongue. [25]

Lifraumeni syndrome, is an autosomal dominant condition and is associated with higher incidence of laryngeal carcinomas.

Bloom syndrome, which is an autosomal recessive disorder is associated with various carcinomas in young population. In head and neck region, they have increased incidence of carcinoma of epiglottis, pyriform fossa and larynx. [26] In immuno-compromised patients due to HIV(Human immune-deficiency virus)/organ transplants, there is increased incidence of oral malignancies. Carcinoma of lip along vermilion border are seen in renal transplant patients. [27]

## SYMPTOMS

In case of oral cavity and oro-pharyngeal growth, they present as chronic non healing ulcer, dysphagia, trismus, poor oral hygiene.

In sino nasal mass, they present as polyp, epistaxsis, nasal obstruction, rhinorrhoea, proptosis, facial pain and diplopia

Nasopharyngeal tumors present with unilateral or bilateral lymphadenopathy, serous otitis media, nasal obstruction and epistaxsis. In advanced cases they present with otalgia, hearing loss, head ache and cranial nerve involvement. Paraneoplastic syndromes like pyrexia of unknown origin, hypertrophic osteoarthropathy are seen.

Laryngeal and hypopharyngeal tumors present as hoarseness, cough, dyspnoea, stridor, change in voice quality, foreign body sensation in throat, dysphagia, odynophagia and hemoptysis. [28]

## INVESTIGATIONS

**Direct and indirect laryngoscopy** is needed for finding the origin of tumor. **Flexible endoscopy** is used for inaccessible areas. **USG** is needed for assessing the involvement of lymphnodes in cervical region.**CT** scan is needed to rule out any bony erosion by the mass in sino-nasal and nasopharyngeal tumors. **MRI** is needed to differentiate between a mucous thickening from fluid collections within the sinuses.

## ORAL POTENTIALLY MALIGNANT LESIONS

### Leukoplakia

It was first coined by a Hungarian pathologist Schwimmer during second half of 19th century. He described them as white mucosal patches. It is not a histopathological term and should be used clinically. Its world wide incidence is 2%-3%. It is associated with same risk factors like carcinomas (smoking, tobacco chewing, etc). These white patches are a result of epithelial injury and chronic irritation resulting in changes in thickness of lining epithelium, keratinisation, parakeratosis with changes in lamina propria including fibrosis and vascularity. We have to look for any dysplastic changes in these reparative process so that leukoplakias with premalignant potential can be identified. (29-30)

### Erythroplakia

**Qeyrat** in the year 1911 described the term "Erythroplasia" who saw an erythematous lesion in glans penis and it was premalignant. Later the term "Erythroleukoplakia" was used for leukoplakia with speckled red areas. WHO placed erythroplakia separately as an entity which has fiery red patch appearence. It has high rate of progression to cancer than the leukoplakia. It is seen in areas with thin squamous mucosa like: ventral tongue, retromolar trigone, floor of mouth and palatine arc. Histologically it is charecterised by thin atrophic mucosa with atypical

cells and no significant keratinisation. Underlying lamina propria shows telangiectatic vessels and chronic inflammatory cells. [31]

**Oral Submucosal Fibrosis**

In India were chewing of betel quid, eating pan masala is common this lesion is more prevelant among the adults. It commonly affects the lip, buccal mucosa, retromolar trigone and soft palate. Early lesions produce mottled appearance. Later lesions demonstrate fibrous bands running around the lip or vertically in mucosa and produces symptoms like trismus. They have a burning sensation which is aggrevated by the spicy foods. Even after cessation of chewing the betel quid there is no reversibility of fibrosis. One fourth develop leukoplakia. Histologically early lesions have dense inflammatory collections with predominent eosinophils and increased vascularity. Later lesions have less inflammatory cells, more fibro-collagen bundles. Any dysplastic changes in epithelium should be looked for. [32-33]

**Dyskeratosis Congenita**

It is an inherited disorder, mostly inherited as an X linked recessive disorder. It is also called as Zinsser-Engman-Cole syndrome, were there is inherited defect in DNA repair system. It can also be inherited as autosomal dominant or as recessive disorder. DKC 1 gene is commonly mutated in X linked disorder. TERC gene is mutated in autosomal

dominant form. It has a clinical triad of dystrophic nails, cutaneous hyperpigmentation and mucosal leukoplakia. It also causes loss of hematopoietic precursors causing pancytopenia. Clinically the patients have esophageal webs, dental caries and genitor-urinary defects.

**Smokeless Tobacco Keratosis**

It is commonly seen in areas like mandibular vestibule were the tobacco leaves are commonly placed. These lesions usually disappear after cessation of chewing tobacco. Histopathologically they show surface parakeratosis, increased intracellular edema in superficial cells and can reach upto parabasal layer. [34]

**Lichen Planus**

It is an auto-immune mucositis and is different from the skin condition. It is common in females with M:F ratio 1:2. Buccal mucosa is most commonly involved. It can also affect other areas like, gingiva, hard palate and lingual dorsum. Clinically it is seen as reticulated web like pattern of slightly raised white keratotic lines. Histopathologically is is charecterised by hyperkeratosis with degeneration of the basal layer, loss of rete ridges with saw tooth like tips of rete with dense chronic inflammatory cells. A subepidermal bulla is seen in some cases. Only in certain subtypes like atrophic / erosive type lichen planus undergo malignant transformation. [35-36]

**Actinic Keratosis**

It is also called as solar keratosis. It is seen in older and middle aged adults in sun exposed areas like face, dorsum of hand, scalp and in vermilion border of the lip. These are less than 1cm and clinically they are erythematous with scales. Transformation to squamous cell carcinoma is seen in 0.5% to 2% of cases. Histopathologically there are five subtypes. They are, hypertrophic, atrophic, bowenoid, acantholytic and pigmented. They show hyperkeratosis, mild parakeratosis, disordered maturation of the epithelium along with nucleomegaly, nuclear atypia, suprabasal mitotic activity and dyskeratosis. In acantholytic type, there is cellular changes which are then followed by acantholysis, so it is called as secondary acantholysis. Bowenoid subtype is similar to bowen disease and has features of carcinoma insitu. In all the subtypes the superficial dermis show dense inflammatory cell infiltrate consisting predominantly of lymphocytes and few plasma cells. [37]The remaining potentially malignant lesions of oral cavity have less chance of conversion to malignant squamous cell carcinoma.

## PRECURSOR LESIONS

Intra-epithelial dysplasia can be keratinizing or non-keratinizing type. In uterine cervical cancers non-keratinizing dysplasia are common

whereas in upper aero-digestive tract keratinizing type dysplasia is more common.

**In earlier time three tier grading system followed:**

| | |
|---|---|
| **Mild dysplasia** | : changes are confined to the basal third of the epithelium |
| **Moderate dysplasia** | : confined to the middle third of the epithelium. |
| **Severe dysplasia** | : changes are confined to whole thickness of the epithelium. [38-39] |

**Recently a two tier grading is being followed:**

**Low grade intraepithelial dysplasia**( includes mild dysplasia)

**High grade intraepithelial dysplasia**(include moderate and severe dysplasia)

To say as dysplasia both cellular and architectural abnormalities are taken into account.

**Architectural Abnormalities Include**:

Irregular epithelial stratification, elongated rete ridges which extend in a downward fashion into underlying stroma. There is loss of maturation with increase in cellularity in the superficial epithelium. Crowding of cells with loss of polarity is seen in the basal zone to as

much as the whole epithelium. All these often occur in the presence of surface keratinization.

**Cellular Abnormalities Include**:

Variation in cell size & shape, abnormal variation in nuclear size (anisonucleosis), abnormnal variation in the nuclear shape (nuclear pleomorphism), increased nuclear to cytoplasm ratio, nuclear hyperchromasia, prominent nucleoli, increased mitotic activity seen in the mid- and upper portions of the surface epithelium, apoptosis and abnormal keratosis like dsykeratosis/ paradoxical maturation seen in individual cells or with keratin pearls in elongated rete ridges / in the basal zone epithelium.

The **cut off point for low grade and high grade dysplasia** according to **Kujan O et all**, is four architectural and five cytological changes, irrespective of the level of dysplasia seen within the epithelium. According to **Nankivell P et al,** the **cut off point** is four architectural and four cytological changes.

The role of human papilloma virus in dysplasia of UADT is less and occur mostly in men, in ventral tongue with presence of koilocytic changes and non-keratinizing histological features.

## SITE SPECIFIC SQUAMOUS CELL CARCINOMA FEATURES

### Sino Nasal Region

Malignancies in sinonasal region forms 3% of Upper aero-digestive tract tumors. Squamous cell carcinomas represents 65% of total malignancies in sino nasal region. [40] Most common site of SCC in sino nasal region is maxillary sinus (55%-60%) and least common site is nasal vestibule, sphenoid and frontal sinuses (1%). [41]

**Keratinizing** squamous cell carcinoma, is charecterised by eosinophilic cytoplasm indicating the individual cell keratinization and intercellular bridges. They are graded into 3 types: well differentiated, moderately differentiated and poorly differentiated based on degree of squamous differentiation and nuclear pleomorphism.

**Non- keratinzing** squamous cell carcinoma. It is also called as schneiderian / transitional cell / cylindrical cell carcinoma. It is charecterised by ribbon like growth pattern with cells are immature appearing and have limited to no keratinization. The basal like cells show peripheral palisading. [42] About 30-50% are associated with high risk HPV infection. It has little or no desmoplastic reaction. There is no grading system for this variant. And is more radiosensitive and carries good prognosis than the keratinizing type. [43]

**Sarcomatoid** squamous cell carcinoma. It is also called as spindle cell squamous cell carcinoma. It is a rare carcinoma constituting < 5% of sinonasal tumors. They are always HPV negative. Smoking and radiation are the common risk factors. It is charecterised by haphazard arrangement of tumor cell with areas of transition to pleomorphic spindle shaped cells. About 7%-15% show heterologous mesenchymal differentiation. Epithelial differentiation must be deomonstrated to say as sarcomatoid carcinoma. [44-45]

**Lymphoepithelial** carcinoma is a variant of squamous cell carcinoma and is morphologicaly similar to nasopharyngeal non-keratinisng carcinoma. It is rare. Only 40 cases are reported in sino-nasal region. [46] More than 90% are associated with EBV infection. Mostly paranasal sinuses are the common site. Histologically it is charecterised by syncytial aggregates, trabecular cords and dyscohesive sheets of tumor cells, intermingled with variable number of lymphocytes and plasma cells. They are positive for EBV encoded small RNA (EBER), Cytokeratin 5/6, p63 and p40. [47]

**Nasopharyngeal Region:**

Nasopharyngeal carcinomas represents 3.7% of total upper aero-digestive tract tumors. Age incidence is 10 -20 years and 40- 60years. [48-49] Most common site is fossa of rosen muller. One of the feature of

nasopharyngeal carcinomas is its propensity for distant meatastasis, The common sites are bone, liver, lung and distant nodes. Jugulo digastric node is the most common node of diatant metastasis.Squamous cell carcinoma in nasopharynx is of **3 types**: Keratinizing, non-keratinizing and basoloid squamous carcinomas.

**Keratinizing** nasopharyngeal squamous cell carcinoma is similar to conventional squamous cell carcinoma of other sites in upper aero-digestive tract. They form 1/4$^{th}$ to ½ of the total nasopharyngeal SCC. They are radio resistant and show less chance for nodal or distant metastasis.

**Non-Keratinizing carcinomas are subdivided into**

I. **Differentiated** subtype show a plexiform and papillary growth patterns with well defined cell borders. Occasional keratinization or intercellular bridges are seen. The cells are smaller than those in undifferentiated subtype, low N:C ratio and indistinct nucleoli.

II. **Undifferentiated** carcinoma is similar to lymphoepithelial carcinoma of sino nasal region. They have syncytial arrangement of tumor cells with individual cell have scant cytoplasm and pleomorphic vesicular nuclei with prominent nucleoli. Cell margins are indistinct and are associated with a

lymphoid stroma. There is no prognostic importance in sub typing non keratinizing squamous cell carcinomas.

**Basaloid squamous** cell carcinoma, is a rare variant in nasopharyngeal region. It is charecterised by nests and lobules of basaloid appearing tumor cells with peripheral palisading of tumor cells in nests seen. Abrupt keratinization are seen focally and with comedo necrosis in centre of some nests are also seen.

Epstein barr virus is present in all types of nasopharyngeal squamous carcinomas and it can be demonstrated by in situ hybridization for EBV encoded small RNA (EBER). [50]

**Oral Cavity with Tongue**

Squamous cell carcinoma of lip forms 10% - 35% of the upper aero-digestive SCC, upper lip is being the most common site. Sun exposure is the most common cause of lip carcinomas. More than 80% tumors does not metastise to lymphnode. Perineural invasion with tumor spreading along the mental nerve into mandible can be seen. Squamous cell carcinoma of oral cavity most commonly affect the tongue, floor of mouth, gingival, all these contributes more than ½. [51] **Squamous cell carcinoma variants of oral cavity includes:** conventional, verrucous, acantholytic, spindle cell, papillary, basaloid, carcinoma cuniculatum, lymphoepithelial and adenosquamous. [52]

**Carcinoma cuniculatum** is a well differentiated type of squamous cell carcinoma of oral cavity. It arises from the mucoperiosteum causing destruction locally and it has a burrowing invasion into the underlying structures. Histologically it resembles verrucous carcinoma and carries good prognosis. [53]

**Acantholytic** squamous cell carcinoma, also called as adenoid SCC. Most common site is lip and then oral cavity. Histologically the tumor cells in areas undergo acantholysis resulting in pseudoglandular pattern. [54] Other types have same features as seen in others sites of the upper aero-digestive tract.

**Oropharynx region**

Squamous cell carcinoma of oropharynx contributes upto 20% - 45% of the upper aerodigestive tract tumors. [55-56] Carcinoma of base of tongue usually present as metastasis to neck nodes in 70% of cases even in tumors with T1 stage. Carcinoma of tonisllar area and soft palate present with severe dysplasia of the lining epithelium with invasion seen multifocally. These tumors metastasie to nodes and present with cystic degeneration of the node. Tumors of soft palate has less chance for nodal metastasis. Carcinoma of posterior pharyngeal wall usually has no symptoms in early stage, so nearly 60% -80% of them are discovered during stage T3 / T4. Human papilloma virus as a causative agent in

squamous cell carcinoma of oro-pharynx is well eshtablished fact and we classify the SCC in this area into:

Squamous cell carcinoma HPV positive

Squamous cell carcinoma HPV negative

**HPV positive squamous cell carcinoma** have features of non – keratinizing squamous cell carcinoma with no dysplasia of the lining epithelium. Tumor cells are arranged in nests with surrounding lymphoid stroma. Areas of keratinization is not seen mostly. Variants like papillary, lymphoepithelial, adenosquamous, sarcomatoid and small cell carcinomas are seen.

**HPV negative squamous cell carcinoma** has features similar to conventional SCC. [57-58]

**Larynx Region**

Laryngeal carcinomas forms 10% - 30% of the upper aero-digestive tract malignancies. Supra glottic area contributes 30%-35% of the laryngeal tumors. Predominant site is pre- epiglottic space. Glottic tumors represent 60%-65% of the laryngeal tumors. Predominant site in glottic region is the anterior third of the vocal cord. Subglottic tumors forms less than 5% of the laryngeal tumors. Mostly tumors arise from 1st tracheal cartilage and lower border of glottis. Transglottic carcinomas are

laryngeal tumors arise from glottis and and invade the supraglottic region. They form less than 5% of the laryngeal tumors. They have aggressive clinical course and more lymph node metastasis. [58]

**Squamous cell carcinoma variants of larynx include**:

Conventional, verrucous carcinoma, basaloid squamous, papillary, spindle cell, lymphoepithelial and adenosquamous carcinomas.

**Verrucous carcinoma** is also called as ackerman tumor. Larynx is the 2nd most common site of occurrence. True vocal cords are the common site in larynx. It is mostly not associated with HPV. It is a well differentiated SCC and is charecterised by thickened, papillomatous projections and invagination of well differentiated squamous epithelium and marked superficial keratinization. They lack cytological atypia and bulbous pushing border with the superficial stromal interface. There is dense lymphocytic infiltrate seen at its interface. [59]

**Papillary** squamous cell carcinoma are rare type. It form 0.5% of all laryngeal tumors, with male predominance. Some carcinomas are associated with HPV. Histologically tumor cells are arranged in papillary pattern with true fibrovascular core. It carries good prognosis due to low stage at presentation. [60]

**Adenosquamous** carcinoma frequently affects the larynx in upper aero-digestive tract. It is charecterised histologically by squamous and

adenocarcinoma areas. Its origin from surface can be identified by the dysplastic changes in the surface. Adenocarcinoma areas like tubules / cribriform pattern are seen deeper in the part of the tumor. Mucin can be seen inside the glands or intracellularly. It goes for nodal metastasis in 75% cases and carries poor prognosis than the conventional squamous carcinoma.

### Hypopharynx region

Carcinoma of hypopharynx carries poor prognosis than other sites in upper aero-digestive tract, since it has high lymphatic drainage, unrestricted growth, more chance for invasion into deeper structures even with intact mucosa. Among the various sites, pyriform fossa contributes 65% – 85% of hypopharynx carcinoma. Postcricoid area contributes lesser with 5%-15%. Most present in advanced stage with nodal metastasis. Level II, III cervical nodes are commonly involved. The squamous cell carcinoma variants are same as seen in larynx.

## OTHER EPITHELIAL TUMORS ARISING FROM VARIOUS SITES OF UPPER AERO-DIGESTIVE TRACT ARE:

### SINO NASAL REGION

### Sinonasal Undifferentiated Carcinoma

It is a rare carcinoma forms 2%-3% of the tumors of the sino nasal region. It has bimodal age population i.e, in teens and 50 -60yr of age. If

EBV and HPV are detected then sinonasal undifferentiated carcinoma is not likely. [62] It usually show invasion into the adjacent structures. Nodal metastasis is rare. Hitopathologically, the tumor shows sheets, trabeculae and lobules of malignant cells with large round nuclei, varying amounts of cytoplasm and defined cell borders. Nuclei vary from hyperchromatic to vesicular with prominent nucleoli. Mitoses, and necrosis are frequent.

It is positive for pancytokeratin (AE1/AE3), CK 8, 18, NSE, chromogranin and synaptophysin. [63]

**Nut Carcinoma**:

It is a poorly differentiated carcinoma that shows NUT gene (Nuclear protein in testis) rearrangement. It is rare in UADT and seen in all age groups with slight female preponderance. [64] More than 60% tumors occur in the nasal cavity and paranasal region. It has no correlation with smoking/ HPV/EBV/ other environmental factors. The diagnosis depends on demonstration of the NUT gene rearrangement by using NUT monoclonal antibodies, which shows nuclear positivity. It also carries poor prognosis. [65 – 66]

**Sinonasal Neuroendocrine Carcinoma**:

It is a high grade carcinoma which has morphological and immunohistochemistry features of neuro-endocrine tumor. It is further subdivided into small and large cell neuroendocrine carcinoma. It forms

3% of the sinonasal tumors, common in ethmoid sinus. Mean age is 49 – 65 years for large cell and 40 – 55 yr for small cell neuroendocrine carcinomas. It is made of small to medium sized cells / large cells in case of large cell neuroendocrine carcinoma with scant cytoplasm, hyperchromatic nuclei, granular chromatin and indistinct nucleoli. [67] Nuclear moulding, necrosis and apoptosis are seen. They grow in sheets, nests and organoid pattern with palisading and rosette pattern. Often there in perineural and lymphovascular invasion. It is positive for neuroendocrine markers like chromogranin, synaphtophysin, CD 56 or neuron specific enolase, LMW CK. EMA show dot positivity. [68]

**Adenocarcinoma**

In sinonasal region, it is of 2 types: Intestinal type and Non-intestinal type.

Intestinal type adenocarcinoma:

It is rare tumor of the sinonasal region. Seen in those exposed to wood and leather dust industries. Mostly seen in $6^{th}$ and $7^{th}$ decade of the life. Mostly seen in lateral wall of nasal cavity and in ethmoid sinuses. Histopathologically it shows papillary and tubular pattern with columnar lining. 75% of the tumor has this pattern( similar to the adenicarcinoma of the intestine), 25% can have mucinous or signet ring like appearance. [69] Tumor cells show loss of polarity with hyperchromatic enlarged cigar

shaped nuclei. Papillary variant is graded into well, moderately and poorly differentiated types based on the arrangement of tumor cells into glandular pattern, in solid sheets and nests. Mucinous type is graded into moderately (alveolar) and poorly differentiated. Signet ring type behaves aggressively. It is positive for Cytokeratin 20, CEA and variable positivity for Cytokeratin 7, MUC2, CDX2 and villin. [70]

Non-intestinal type adenocarcinoma:

It is of two types:

1. Sero-mucinous/low grade Non intestinal adenocarcinoma (LG-Non ITAC )
2. High grade non intestinal adenocarcinoma(HG –Non ITAC)

No specific etiology is seen, although some high grade NIAC are seen with HPV infection. Mostly affect the middle turbinate.

Histpathologically : LG-Non ITAC show tubular and papillary pattern with complex cribriform architecture. Mitosis and necrosis are absent.

Histopathologically: HG –Non ITAC have predominantly solid and occasional glandular pattern with infiltrative pattern of growth. Areas of necrosis with more mitotic figures are seen. Some tumors are composed of tumor cells with clear cytoplasm similar to the metastatic deposits of renal cell carcinoma. They are called as renal cell like carcinoma. [71]

LG NonITAC is positive for cytokeratin 7. Other markers like CK 20, CDX2, MUC2, p63/p40 are mostly negative.

Some show positive reaction with SOX 10, DOG1 / S100.

HG Non ITAC show focal positivity for neuroendocrine markers.

(Both LG & HG have intracytoplasmic / intrauminal mucin demonstrated by PAS and diastase resistence.)

Renal cell like carcinoma –Express CD 10, Carbonic anhydrase IX, beta catenin and p53. Negative for PAX 8.

**Teratocarcinosarcoma**

It is also called as terato carcinoma/ malignant teratoma. It has features of teratoma with carcinosarcoma and lack any malignant germ cell component. It is common in males and nasal cavity is the most common site. Its thought to arise from the neuro-epithelium of the olfactory membrane. [72] Histologically it has epithelial, mesenchymal and neural components. Epithelial component consists of keratinizing, non-keratinizing squamous cells, columnar cells lining the ductal or cystic spaces. Mesenchymal component has fat cells, spindle shaped fibroblast and myofibroblast, rhabdoid cells, cartilage and bone. Carcinoma either in the glandular or squamous cells is seen with the

background shows immature neural elements / neurofibrillary background. It is a highly aggressive tumor. [73]

**Sinonasal Papilloma**

It is of 3 types: Exophytic, Inverted and Oncocytic papilloamas.

Exophytic Papilloma:

It is also called as Everted/ Fungiform/Transitional cell papilloma/ Ringertz tumor. Age group is 20 – 50 years. Male population is affected more. It may be associated with high risk HPV infection. It is composed of papillary fronds with true vascular core lined by multiple layered epithelium (5 – 10 cell thick). Epithelium can be squamous/ respiratory/ transitional type. Scattered mucocytes are seen. With infection stroma show inflammatory cells and edema. Malignant transformation in this papilloma is rare. [74]

Inverted Papilloma:

It is more common in nasal cavity and medial wall of the maxillary sinus. Age group is $5^{th}$ – $6^{th}$ decade. Male are commonly affected. Exposure to organic solvents and low risk HPV are risk factors for development of inverted papilloma. Histologically it shows multiple inverted growth of the epithelium into the underlying stroma. Epithelium is non-keranitinizing squamous epithelium / transitional epithelim 5 – 30

cells thick. [75] They also show trans migrating neutrophils. Mitosis is seen in the basal layer of the epithelium. Malignant transformation is seen in 2% - 27% of cases.

Oncocytic Type:

It is also called as cylindrical cell / columnar cell papilloma. It has equal sex distribution with age more than 50years. Usually it occurs in lateral wall of nasal cavity. It has both exophytic and endophytic growth pattern, lined by multilayered(2-8 cell thick) columnar cells with oncocytic features (cells have fine granular eosinophilic cytoplasm due to more mitochondria). The epithelium shows numerous cysts filled with mucin and neutrophils. It rarely undergo malignant transformation. [76]

**Respiratory Epithelial Adenomatoid Hamartoma (REHA):**

It is a benign lesion, also called as glandular hamartoma. It shows male preponderance and peak age incidence ranges from $3^{rd}$ – $8^{th}$ decade of life. Most of cases occur in posterior nasal septum. Histologically, there is overgrowth of glands in continuity with the respiratory epithelium. Scattered goblet cells are also seen in the lining glands. The glands are surrounded by a thick eosinophilic basement membrane. Glands may be cystically dilated/ atrophic with variable hyalinization in stroma. [77-78]

## NASOPHARYNGEAL REGION

### Nasopharyngeal Adenocarcinoma

It is also called as low grade papillary adenocarcinoma. It forms less than 1% of the nasopharyngeal tumors. Mean age at which tumor occurs is 10 – 69 years and has no sex predilection. Histologically they show complex papillae lined by single layer of columnar epithelium with eosinophilic cytoplasm. In some cases psammomatoid calcification are seen. Mitosis / necrosis is rare.

Immunohistochemistry, it express EMA, CK5/6 and CK 7. Few cases show positivity for TTF1 but are negative for thyroglobuline. [79]

### Ectopic Pituitary Adenoma

It forms less than 3% of the nasopharyngeal tumors. It is a benign anterior pituitary gland tumor. It has a wide age with mean age of 55 years with female preponderance. Some may present with endocrinopathic manifestations like galactorrhoea and cushing syndrome. It is a submucosal epithelioid cell neoplasm, with cells have eosinophilic granular cytoplasm, round nuclei and fine dispersed chromatin. They are arranged in trabecular and organoid growth pattern in a highly vascularized and collagenized stroma. [80]

Immunohistochemistry: Positivity for chromoganin, synaptophysin, CD 56, S100 protein, and dot like positivity of cytokeratin.They also show positivity for pituitary hormones. But 20% cases don't secrete any hormones and show no positivity for hormones( Null cell adenomas).

**Craniopharyngioma**

It is also called as pituitary adamantinoma and can occur extracranially in nasopharynx. They have a cystic component that contains fluid called machine oil fluid. It is derive from rathke cleft. Hisologically it is of 2 types: adamantinomatous and papillary type. In adamntinomatous type, basaloid appearing cells surround the central loose stellate like cells, with areas of wet keratin and calcification are seen. They are associated with CTNNB1 mutations. In papillary type sheets of mature squamous epithelium arranged in papillary pattern with fibrovascular core are seen. They are associated with BRAF mutations. (81)

**ORAL CAVITY WITH OROPHARYNX**

**Papillomas**

They are common and occur in 3$^{rd}$ to 5$^{th}$ decade. Low grade HPV virus ( 6, 11) is the most common cause. Most common site is soft palate, tongue and gingival. These are pedunculated or sessile verrucous lesions. Histologically it consists of stratified squamous epithelium arranged in

papillary pattern with vascular core and variable degree of parakeratin / orthokeratinization. Koilocytic changes are rare.

**Condyloma Acuminatum**

It is similar to the anogenital condyloma acuminata(CA). It is transmitted by sexual contact. Young adults are most commonly affected. In oral cavity it can occur in soft palate and frenulum. Clinically these are larger than squamous papilloma. HPV 6, 11 is found to be the causative agents in most of the cases. Histologically, they show exophytic growth with hyperplastic squamous proliferation with fibrovascular core and a broad base. Keratinocyte maturation is maintained here. Papillae is more broad and have blunt ends, rete ridges are short, straight and bulbous and have koilocytic changes. Malignant changes in oral condyloma acuminatum is very rare. (82)

**Verruca Vulgaris**

It is common in skin and can occur in oral cavity as a result of auto-innoculation. It has slight male preponderance seen in $3^{rd}$ – $4^{th}$ decade. Lips, anterior tongue, hard palate and gingiva are the common sites to be involved. HPV 2, 4, 40 57 are the etiological agents. Histologically, they are seen as exophytic papillary lesions that show thick layers of orthokeratin and rete ridges coverge to the centre. Granular layer shows keratohyaline granules and koilocytic changes. (83)

## LARYNX AND HYPOPHARYNX REGION

### Neuroendocrine Tumors

They are classified into well differentiated, moderately differentiated and poorly differentiated carcinomas based on pleomorphism of the tumor cells and mitotic acitivity. (84)

### Well differentiaed Carcinoma

It is also called as carcinoid/neuroendocrine carcinoma grade I . It is more common in supraglottic region. Men in middle age are affected. Tobacco use is a risk factor. Histologically, tumor cells have eosinophilic cytoplasm with round nuclei and salt & pepper chromatin. They are arranged in trabeculae, cords and nests. Mitosis is less than 2 per 10 HP field. In most of cases hormone secretion is not seen. (85)

### Moderately differentiated Carcinoma

It is also known as atypical carcinoid/ neuroendocrine carcinoma grade II. This ia also common in larynx and present clinically as a small polyp. Histologically, they show moderate pleomorphism and mitosis more than 2 per HPF. Apart from the neuroendocrine markers, they also show positivity for calcitonin and variable positivity for TTF-1. (86)

**Poorly differentiated Carcinoma**

It is a high grade malignant epithelial neoplasm which shows neuroendocrine differentiation. It is subdivided into:

Small cell neuroendocrine carcinoma

Large cell neuroendocrine carcinoma

Poorly differentiated carcinoma is more common than the well differentiated type in larynx. Small cell type show nuclear moulding and azzopardi effects. Both subtypes show areas of necrosis and mitotic avtivity of more than 2 per 10 HPF. [87]

**Salivary Gland Tumors**

In all these regions of upper aero-digestive tract salivary gland tumors can occur, they include:

Pleomorphic adenoma

Adenoid cysctic carcinoma

Mucoepidermoid carcinoma

Oncocytic papillary cystadenoma

Polymorphous adenocarcinoma

Salivary gland analage tumor

**Pleomorphic Adenoma**

It is a benign mixed salivary gland tumor. It can arise from the mucinous glands in nasal septum. In nasal cavity they usually have dominant epithelial component. [88]

**Adenoid Cystic Carcinoma &Polymorphous Adenocarcinoma**

It arises from the minor salivary glands, most common site is hard palate of the oral cavity. Both are malignant tumors and has tendency for perineural invasion with destruction of the underlying bone. [89]

**Mucoepidermoid Carcinoma**

It is a distinct tumor which has mucinous, squamoid and intermediate cells forming solid and cystic areas. Parotid is the most common site and minor salivary glands are 2nd common site. It can be graded into low, medium and high grade depending upon the population of three cells. [90]

**Oncocytic Papillary Cystadenoma**

It arises from the minor salivary glands. Most common site is supraglottis, seen in 6th - 7th decade. It can be uni/multicystic lined by oncocytic epithelium and papillary projections. These are benign tumors with less chance for recurrence. [91]

**Salivary gland analage tumor**

It is also called as congenital pleomorphic adenoma. It occurs in posterior nasal septum and is associated with respiratory distress and before birth it is associated with polyhydromnious. Histologically seen as biphasic population of epithelial and myoepithelial cell proliferation. Epithelial component seen as ducts and tubules showing variable keratinization seen in continuity with the surface epithelium. Myoepithelial component consisting of spindle shaped cells with variable hypo and hypercellular areas seen in centre of the polyp. [92]

**PROGNOSTIC FACTORS**

1. Tumor size plays an important role. Any tumor with size less than 2cm carries good prognosis and more than 4cm carries bad prognosis.
2. Site of organ: Hypopharyngeal and subglottic tumors carries worse prognosis in UADT.
3. Lymph node metastasis : Cases with no lymphnode metastasis has good prognosis.
4. Perineural and lymphovascular invasion.

5. Histlogical subtypes: Histological types like verruccous carcinoma, papillary variant of SCC carries good prognosis and baso squamous, adenosquamous variants carry poor prognosis.
6. Invasive front: A simple grading system to evaluate the invasive front of the tumor by taking 5 histopathological features is done: they are shown in following table.

   Each has 4 scoring system and based on the total score by adding the individual score they can be graded.

   A score of 6 to 10 - grade 1, score of 11 to 15 – grade 2 and score of 16 to 20 – grade 3

## GRADING SYSTEM IN INVASIVE MARGIN OF SCC

| Morphology | Score 1 | Score 2 | Score 3 | Score 4 |
|---|---|---|---|---|
| **Degree of keratinization** | More than 50% of cells | 20% to 50% of cells | 5% to 20% of cells | 0% to 5% of cells |
| **Nuclear pleomorphism** | More than 75% mature cells | 50% to 75% mature cells | 25% to 50% mature cells | 0 % to 25% mature cells. |
| **Pattern of growth** | Pushing & well delineated borders | Infiltrating solid cords, bands/ strands | Small group of infiltrating cells | Widespread cellular dissociation / single cells |
| **Inflammatory response** | Marked | Moderate | Slight | None |
| **Number of mitosis per high power field.** | 0 to 1 | 2 to 3 | 4 to 5 | More than 5 |

7. Surgical margins free of tumor infiltration has good prognosis.

8. Metastasis to different organ/site

9. Molecular markers: p53 expression carries poor prognosis. p16 (a surrogate marker for HPV) expression carries good prognosis and better disease free survival rate compared to HPV negative tumors.

# MATERIALS AND METHODS

## STUDY DESIGN

Present study is a prospective study conducted in pathology department, of Madurai Medical College during July 2016 to July 2018 period. Ethical clearance for the study was obtained from the Ethical Committee of Madurai medical college, Madurai.

A sample of 100 cases are taken for this study on histopathological examination with p16 immunohistochemistry.

## INCLUSION CRITERIA

1. All newly diagnosed cases of primary upper-aerodigestive tract squamous cell carcinoma, diagnosed by histopathological examination.
2. Adults of either sex.

## EXCLUSION CRITERIA

1. Patients with carcinomas other than squamous cell carcinomas as demonstrated by histolpathological examination.
2. Patients with carcinomas on/ prior treatment with radiation and chemotherapy.

## METHODOLOGY AND TECHNIQUES

The study material included 100 squamous cell carcinoma cases. (Annexure VI). The clinical and histolopathological features of the cases are recorded from the proforma (Annexure III). The specimens were fixed in 10% neutrally buffered formalin. After adequate fixation the biopsy specimen is processed routinely to obtain multiple 4 to 6 micron thin paraffin sections. Staining is done using hematoxyline and eosin method. (Annexure IV).

## HISTOPATHOLOGICAL EVALUATION

Stained slides are examined under light microscopy. Squamous cell carcinoma are classified as keratinizing / non keratinizing tumors and are further subclassified as verrucous, papillary, acantholytic, etc based on pattern of squamous cell carcinoma. (Annexure VI)

## IMMUNOHISTOCHEMISTRY

In overall specimens of squamous cell carcinoma of UADT, 100 cases are taken and its correlation with HPV was studied by immunohistochemistry using p16 monoclonal antibody. Cases were classified as positive and negative by as follows:

| S.No | Percentage of positive tumor cells | Result |
|---|---|---|
| 1. | Diffuse nuclear and cytoplasmic staining in > 70% of tumor cells | Positive |
| 2. | Membranous / cytoplasmic staining in < 40% tumor cells | Negative |
| 3. | Complete absence of staining | Negative |

## STATISTICS

Fischer's exact test was used to test differences in clinical characteristics, and to test the p16 results between histologic groups. A P value less than 0.05 was considered statistically significant.

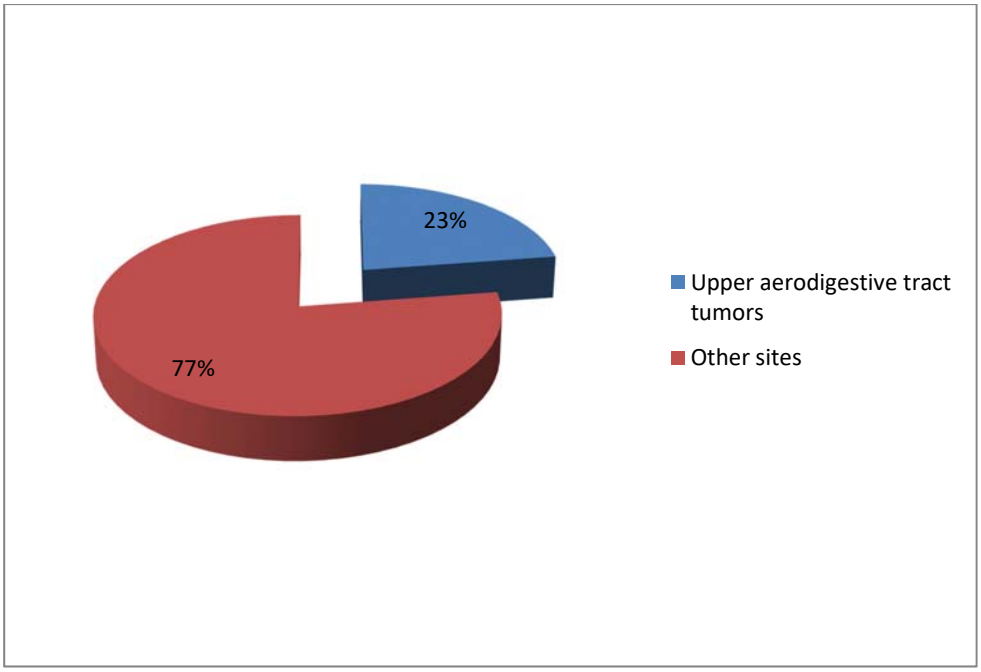
## OBSERVATION AND RESULTS

In our institute, the total number cases diagnosed by small biopsy specimens during the period of july 2016 to july 2018 was 3750 biopsies. In which 850 specimens were from upper – aerodigestive tract. Average incidence of upper aerodigestive tract tumors in this hospital was 23%

**TABLE 1: AVERAGE INCIDENCE OF UPPER AERODIGESTIVE TUMORS**

| TYPE OF SPECIMENS | FREQUENCY | PERCENTAGE |
|---|---|---|
| Upper aerodigestive tract specimens | 850 | 23% |
| Other specimens | 2900 | 77% |
| **TOTAL** | 3750 | 100% |

## CHART 1: AVERAGE INCIDENCE OF UPPER AERODIGESTIVE TRACT TUMORS.

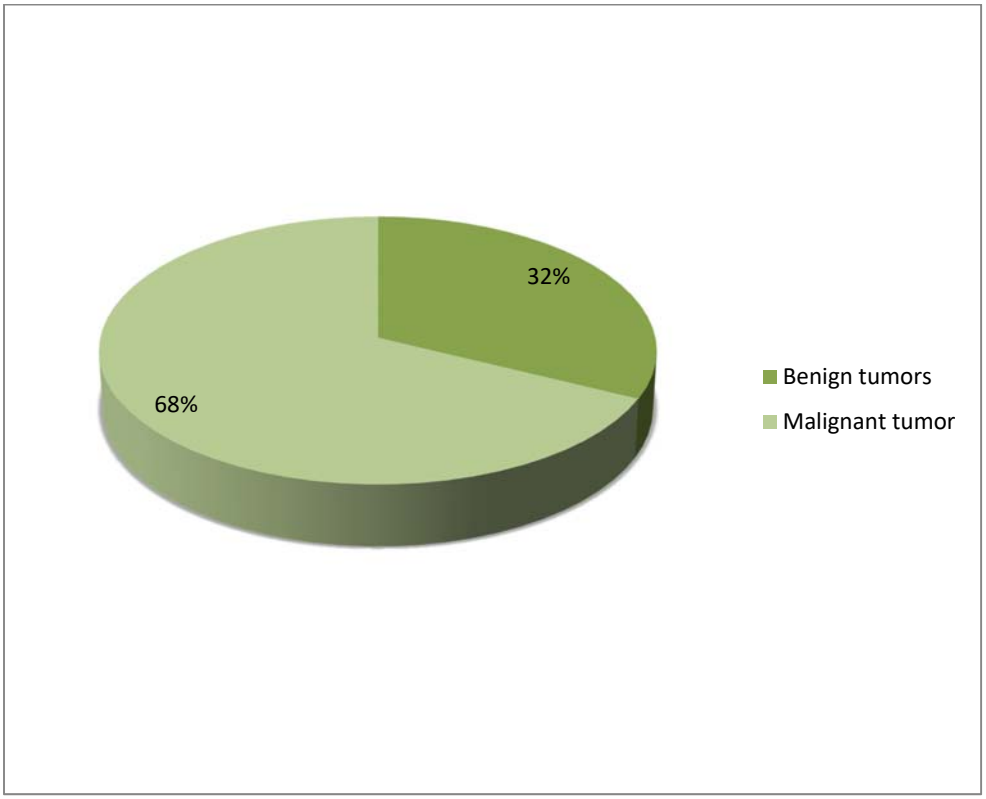



## TABLE: 2 - DISTRIBUTION OF TUMOR ACCORDING TO THEIR BEHAVIOUR

| TYPE | DISTRIBUTION | |
|---|---|---|
| | FREQUENCY | PERCENTAGE |
| Benign | 248 | 32% |
| Malignant | 527 | 68% |
| **Total** | 775 | 100% |

In present study, with the total 850 biopsy specimens of upper aero-digestive tract, 75 specimens were inadequate for reporting. In remaining 775 cases 248 (32%) cases were benign and 527 (68%) were malignant tumors. (Table 2 and Chart 2).

**CHART 2 : DISTRIBUTION OF TUMOR ACCORDING TO THEIR BEHAVIOUR**

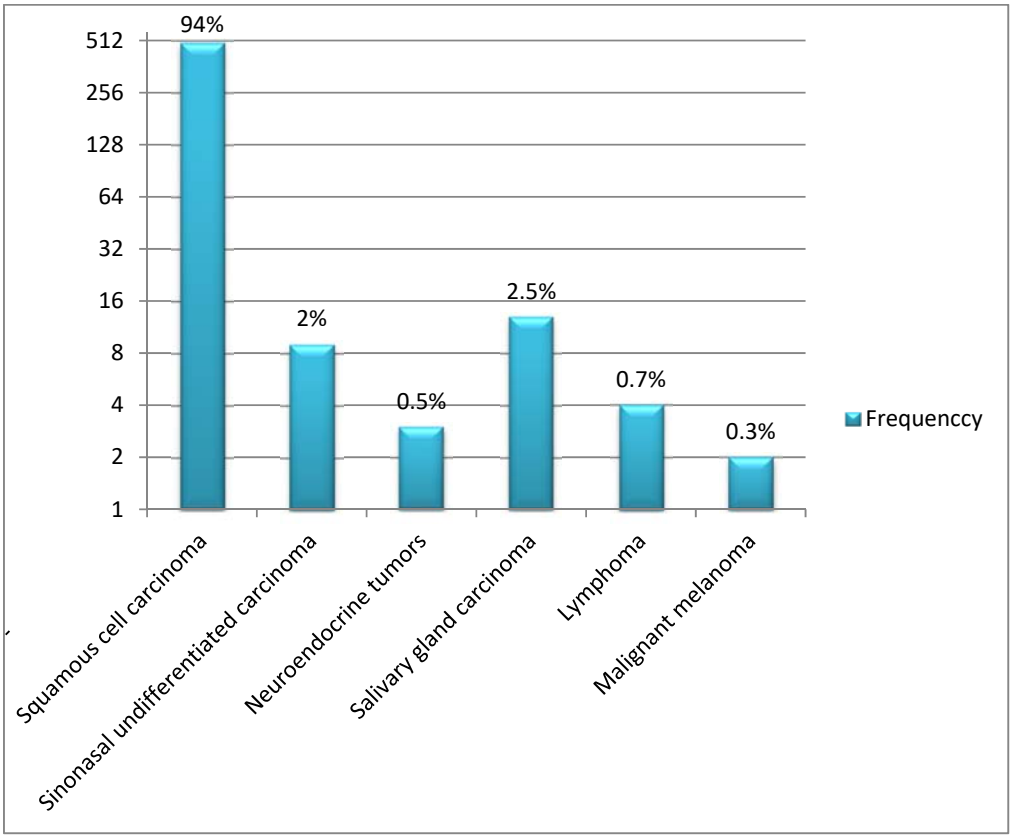


## TABLE: 3 - INCIDENCE OF UPPER AERODIGESTIVE TRACT TUMORS

| TUMORS | NUMBER | FREQUENCY |
|---|---|---|
| Squamous cell carcinoma | 496 | 94% |
| SinonasalUndifferentiated carcinoma | 9 | 2% |
| Neuroendocrine tumors | 3 | 0.5% |
| Salivary gland carcinomas | 13 | 2.5% |
| Lymphoid tumors | 4 | 0.7% |
| Malignant melanoma | 2 | 0.3% |
| **Total** | **527** | **100%** |

The most common tumors of upper aerodigestive tract in present study was squamous cell carcinomas with 94% of total malignant tumors of UADT, 2nd common tumors were the salivary gland carcinomas ( Adenoid cystic carcinoma and polymorphous adenocarcinoma) with 2.5% of total malignant tumors of UADT and a small percentage is contributed by neuroendocrine tumors, lymphomas and malignant melanoma .(Table 3 and chart 3).

## CHART 3: INCIDENCE OF TUMORS OF UPPER AERODIGESTIVE TRACT TUMORS

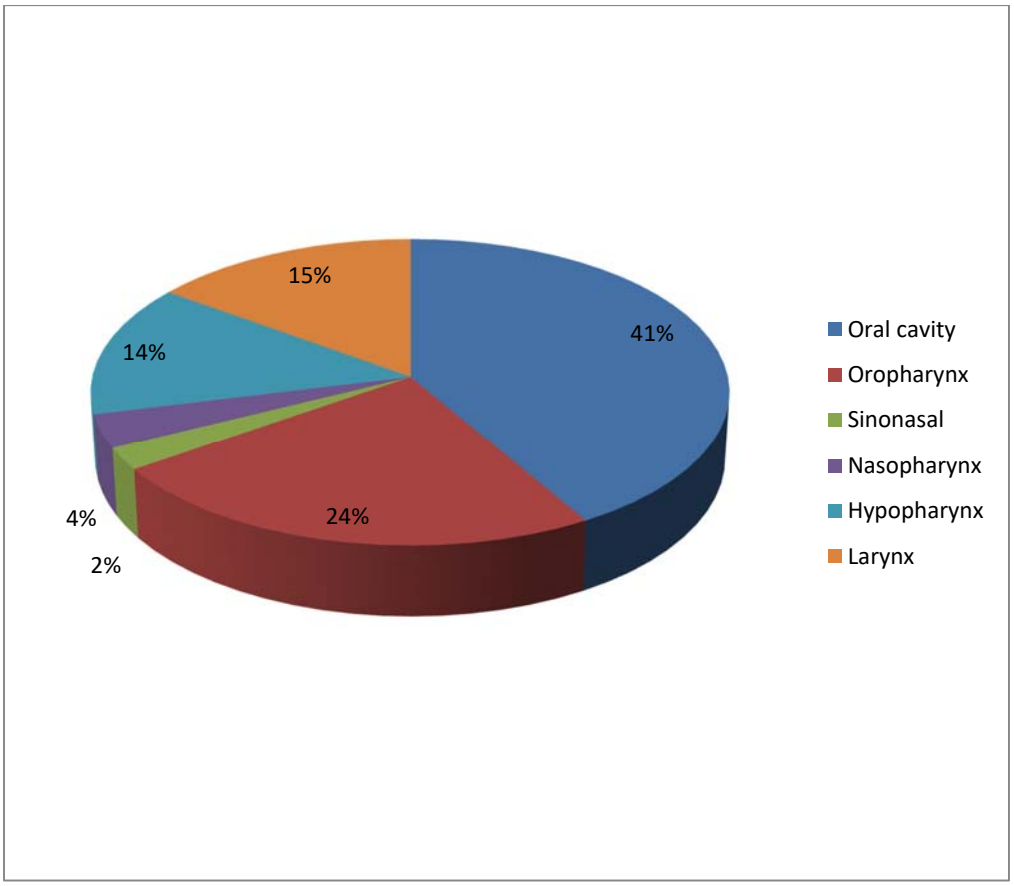


## TABLE:4 – SITE WISE DISTRIBUTION OF TUMORS OF UPPER AERODIGESTIVE TRACT

| SITES | NUMBER | FREQUENCY |
|---|---|---|
| Oral cavity | 199 | 41% |
| Oropharynx | 120 | 24% |
| Sinonasal | 12 | 2% |
| Nasopharynx | 18 | 4% |
| Hypopharynx | 71 | 14% |
| Larynx | 76 | 15% |
| **Total** | **496** | **100%** |

Table 4, chart 4 shows different anatomical site wise distribution of the upper aerodigestive tract tumors in present study. Oral cavity contributes to most of the tumors with 41% and second common site was oropharynx with 24% and sinonasal region forms the least common site and contributes 5% of the total tumors of upper aerodigeative tract.

## CHART:4 - SITE WISE DISTRIBUTION OF TUMORS OF UPPER AERODIGESTIVE TRACT



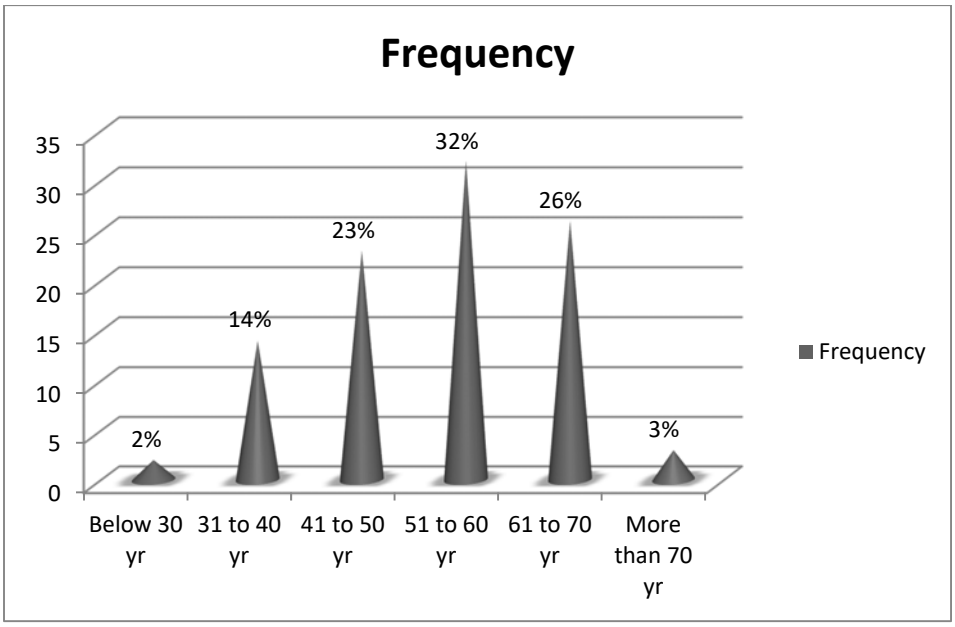

In overall 496 cases of squamous cell carcinoma of upper aerodigestive tract, 100 cases are taken for correlation with human papilloma virus infection and are studied using p16 marker. These 100 cases include both keratinizing and non keratinizing carcinomas.

**TABLE 5: AGE WISE DISTRIBUTION OF SQUAMOUS CELL CARCINOMA OF UADT**

| AGE | FREQUENCY | PERCENTAGE |
|---|---|---|
| Below 30 yr | 2 | 2% |
| 31 to 40 yr | 14 | 14% |
| 41 to 50 yr | 23 | 23% |
| 51 to 60 yr | 32 | 32% |
| 61 to 70 yr | 26 | 26% |
| More than 70 yr | 3 | 3% |
| **Total** | **100** | **100%** |

In present study on SCC of UADT, peak age of incidence was seen beween 51 to 70 years in both males and females. The minimum age of patient was 18 yr and maximum age of patient was 77 yr . (Table 5 and chart 5)

## CHART 5: AGE WISE DISTRIBUTIONOF SQUAMOUS CELL CARCINOMA OF UADT

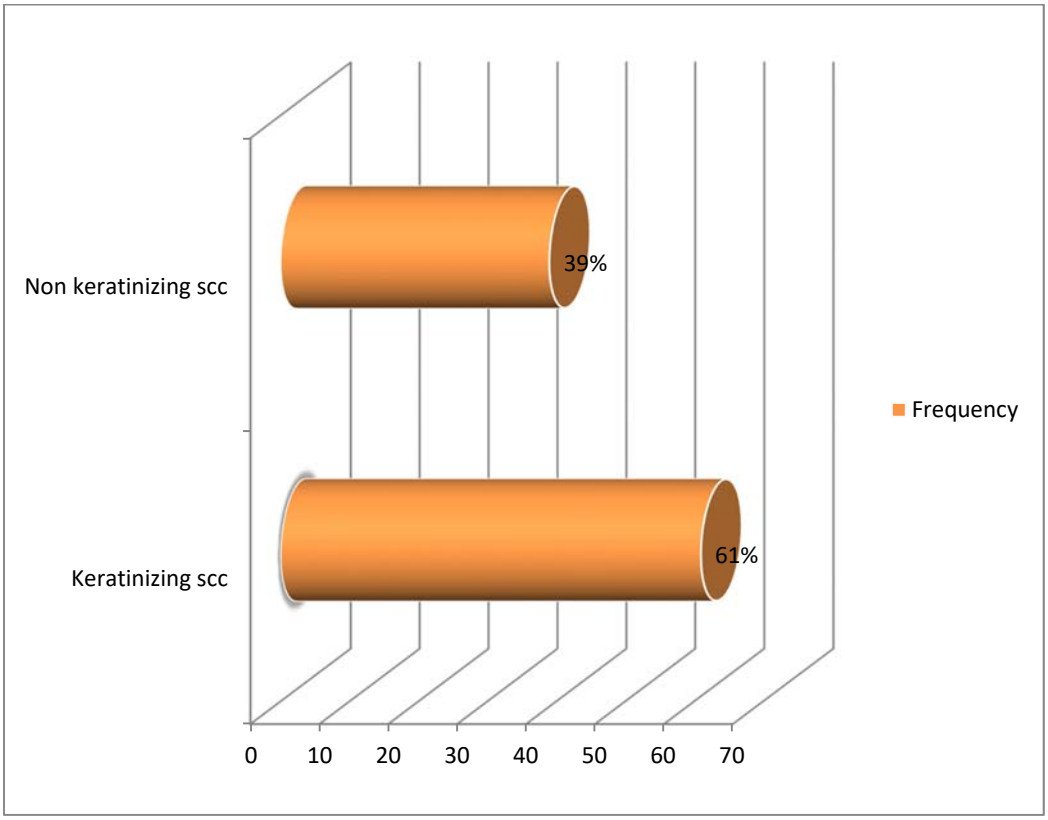



## TABLE 6: INCIDENCE OF KERATINIZING AND NON-KERATINIZING SCC IN UADT

| MORPHOLOGICAL TYPE OF SCC | FREQUENCY | PERCENTAGE |
|---|---|---|
| Keratinizing SCC | 61 | 61% |
| Non keratinizing SCC | 39 | 39% |
| **Total** | **100** | **100%** |

In present study, SCC of UADT were histologically classified into keratinizing and non keratinizing carcinomas. In total of 100 cases, keratinizng SCC form 61% and non keratinizing SCC contribute 39%. (Table 6 and chart 6)

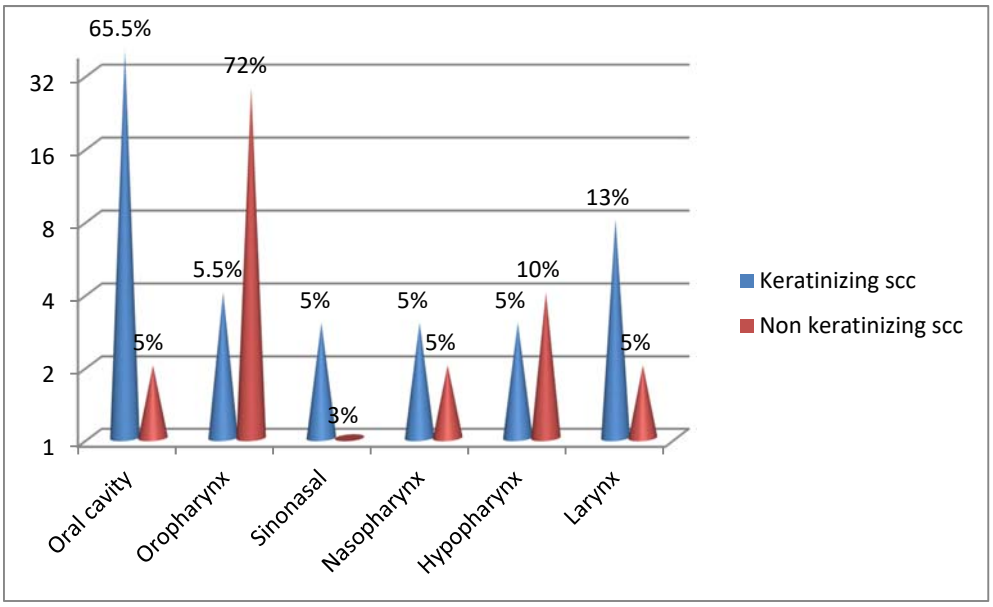
**CHART 6: INCIDENCE OF KERATINIZING AND NON KERATINIIZING SCC IN UADT**



## TABLE 7: DISTRIBUTION OF KERATINIZING AND NON KERATINIZING SCC IN VARIOUS SITES OF UADT

| SITES | KERATINIZING SCC | NON KERATINIZING SCC | TOTAL |
|---|---|---|---|
| Oral cavity | 40 | 2 | 42 |
| | 65.5% | 5% | |
| Oropharynx | 4 | 28 | 32 |
| | 6.5% | 72% | |
| Sinonasal | 3 | 1 | 4 |
| | 5% | 3% | |
| Nasopharynx | 3 | 2 | 5 |
| | 5% | 5% | |
| Hypopharynx | 3 | 4 | 7 |
| | 5% | 10% | |
| Larynx | 8 | 2 | 10 |
| | 13% | 5% | |
| **Total** | **61** | **39** | **100** |
| | **100%** | **100%** | |

## CHART 7: DISTRIBUTION OF KERATINIZING AND NO KERATINIZING SCC IN VARIOUS SITES OF UADT




In present study, oral cavity and laryngeal regions showed high incidence of well differentiated & keratinizing type SCC with overall 40 cases (65.5% ) and 8 cases (13%) respectively and other sites: Oropharynx and hypopharyngeal region showed high incidence of non keratinizing SCC with 28 cases (72%) and 4 cases(10%) respectively. (Table 7 and chart 7)

## TABLE8 : INCIDENCE OF DIFFERENT VARIANTS OF KERATINIZING SCC IN UADT

| Site | Conventional Scc | Verrucous | Papillary scc | Acantholytic | Sarcomatoid carcinoma | Total |
|---|---|---|---|---|---|---|
| Oral cavity | 32 | 6 | 1 | 1 | 0 | 40 |
| Oropharynx | 2 | 0 | 1 | 1 | 0 | 4 |
| Sinonasal | 3 | 0 | 0 | 0 | 0 | 3 |
| Nasopharynx | 3 | 0 | 0 | 0 | 0 | 3 |
| Hypopharynx | 2 | 0 | 1 | 0 | 0 | 3 |
| Larynx | 3 | 0 | 3 | 0 | 2 | 8 |
| **Total** | **45** | **6** | **6** | **2** | **2** | **61** |
| **Percentage** | **74%** | **10%** | **10%** | **3%** | **3%** | **100%** |

## CHART 8: INCIDENCE OF DIFFERENT VARIANTS OF KERATINIZING SCC IN UADT

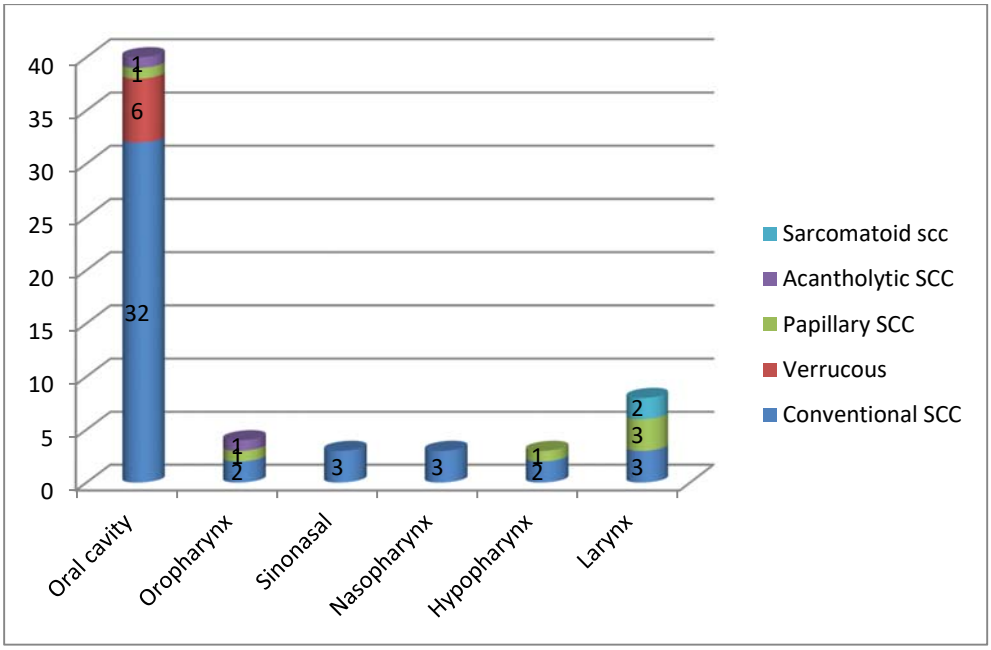


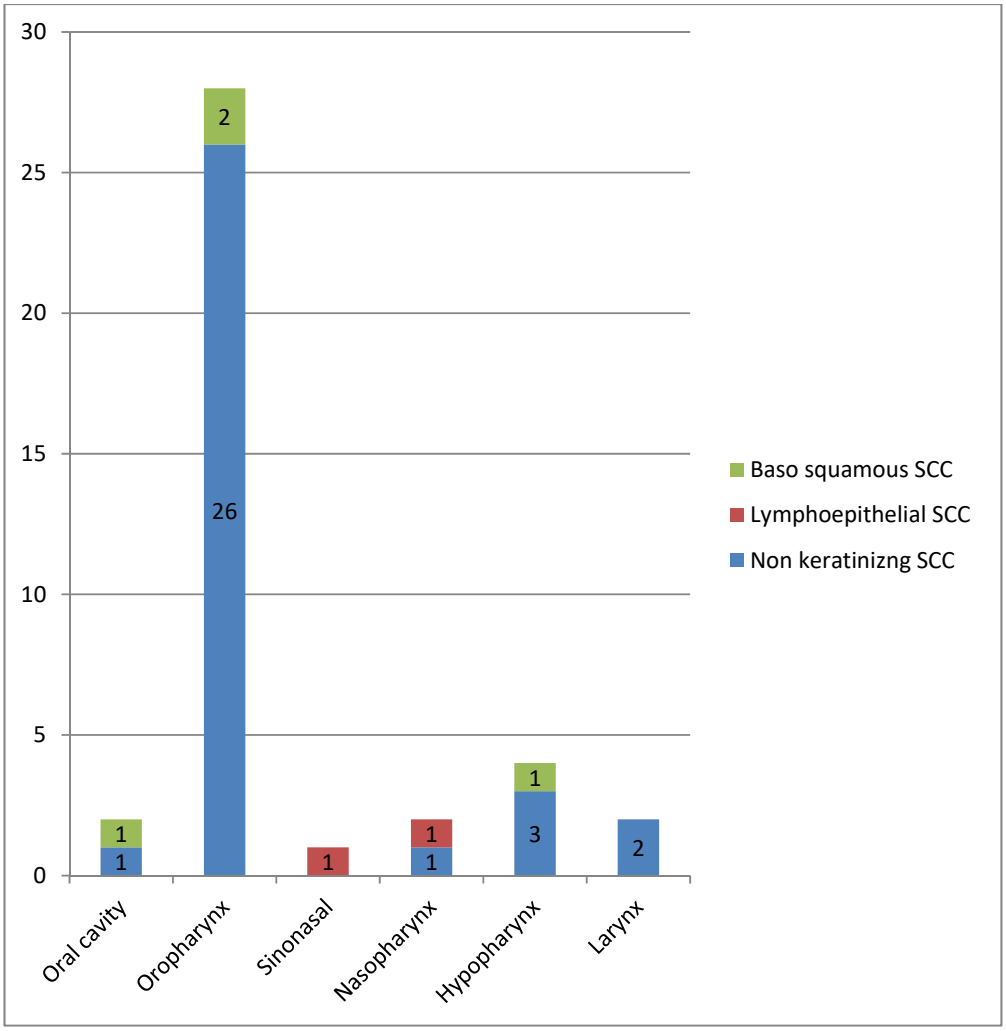

Table 8 and chart 8 shows the different variants of keratinizing SCC in different sites of UADT. Most common variants were conventional and verrucous carcinoma. They were frequently seen in oral cavity with 32 cases and 6 cases. Papillary variant was frequently seen in laryngeal region with 3 cases and rare type acantholytic and sarcomatoid carcinoma were seen occasionally in oral cavity and laryngeal regions.

## TABLE 9: INCIDENCE OF VARIANTS OF NON KERATINIZING SCC IN UADT

| Site | Non keratinizing scc | Lymphoepithelial carcinoma | Basaloid squamous | Total |
|---|---|---|---|---|
| Oral cavity | 1 | 0 | 1 | 2 |
| Oropharynx | 26 | 0 | 2 | 28 |
| Sinonasal | 0 | 1 | 0 | 1 |
| Nasopharynx | 1 | 1 | 0 | 2 |
| Hypopharynx | 3 | 0 | 1 | 4 |
| Larynx | 2 | 0 | 0 | 2 |
| **Total** | **33** | **2** | **4** | **39** |
| **Percentage** | **85%** | **5%** | **10%** | **100%** |

Table 9 and chart 9 shows the variants of non keratinizing SCC in different sites of UADT. This variant of SCC were more common in oropharyngeal region with 26 cases and other variants like basaloid squamous and lymphoepithelial carcinoma were less common constituting 4 and 2 cases of total SCC.

## CHART 9: INCIDENCE OF VARIANTS OF NON KERATINIZING SCC IN UADT



## TABLE 10: AGE AND SEX WISE DISTRIBUTION OF KERATINIZING SCC

| AGE | MALE | FEMALE | TOTAL |
|---|---|---|---|
| Below 30 yr | 0 | 1 | 1 |
| 31 to 40 yr | 1 | 2 | 3 |
| 41 to 50 yr | 4 | 3 | 7 |
| 51 to 60 yr | 20 | 5 | 25 |
| 61 to 70yr | 19 | 3 | 22 |
| More than 70 yr | 2 | 1 | 3 |
| **Total** | **46** | **15** | **61** |
| **Percentage** | **75%** | **25%** | **100%** |

In present study of age and sex wise distribution of keratinizing SCC showed male predominence with 82% and females with incidence of 18%. Peak age of incidence range from 51 to 70yr and the incidence decreased after 70 yr. Mean age was 58 yr. (Table 10 and chart 10).

## CHART 10: AGE AND SEX WISE DISTRIBUTION OF KERATINIZING SCC

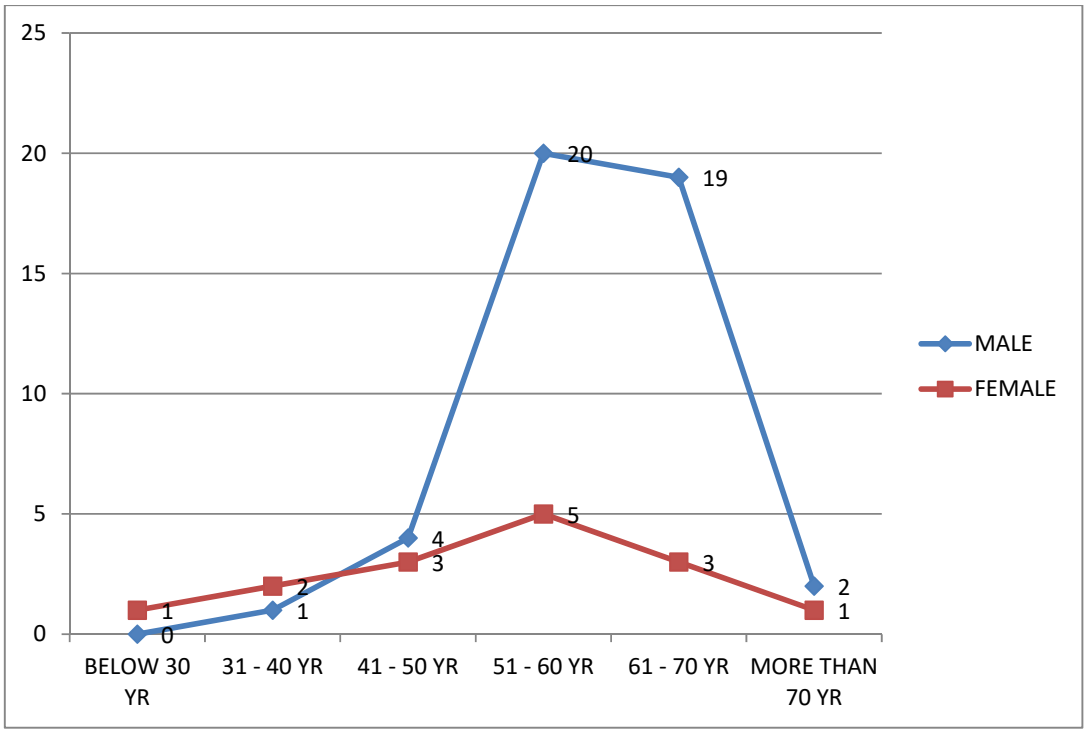


**TABLE11: AGE AND SEX WISE DISTRIBUTION OF NON KERATINIZING SCC**

| AGE | MALE | FEMALE | TOTAL |
|---|---|---|---|
| Below 30 yr | 0 | 1 | 1 |
| 31 to 40 yr | 8 | 3 | 11 |
| 41 to 50 yr | 11 | 4 | 15 |
| 51 to 60yr | 6 | 1 | 7 |
| 61 to 70yr | 4 | 1 | 5 |
| More than 70 yr | 0 | 0 | 0 |
| **Total** | **29** | **10** | **39** |
| **Percentage** | **74%** | **26%** | **100%** |

The age and sex wise distribution of non keratinizing scc in present study also showed a male predominence with 29 cases(70%) and females with 10 cases(30%). Peak age of incidence for both sexes range from 31 to 40yr and the incidence decreased after 50 yr. Mean age was 48 yr. (Table 11 and chart 11)

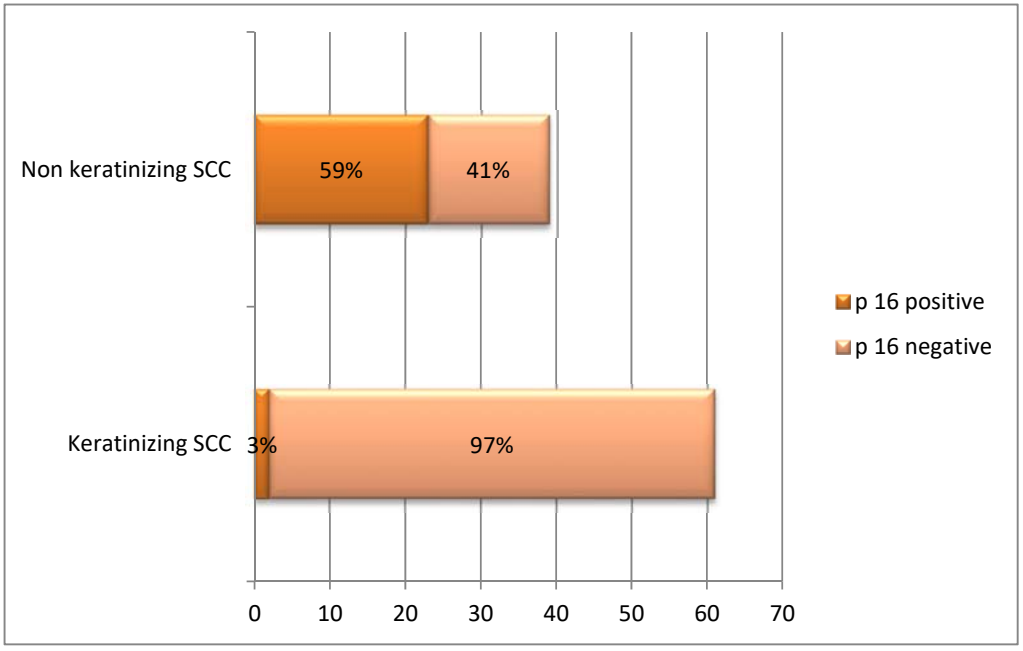
## CHART 11: AGE AND SEX WISE DISTRIBUTION OF NON KERATINIZING SCC

## TABLE 12: p16 IHC IN SCC OF UADT

| TYPE OF SCC | p16 POSITIVE | p16 NEGATIVE | TOTAL |
|---|---|---|---|
| Keratinizing SCC | 2 | 59 | 61 |
| Non keratinizing SCC | 23 | 16 | 39 |
| **TOTAL** | **25** | **75** | **100** |

(Table 12,chart 12)In present study p16 IHC was studied in both keratinizing and non keratinizing SCC of UADT and p16 showed maximum positivity in non keratinizing SCC with 23 cases (59%) and the positive cases in keratinizing SCC were 2 cases (3%).

## CHART 12: p16 IHC IN SCC OF UADT




By fischer's exact test, the p value was calculated and it was less than 0.001. The relationship between histopathological type of squamous cell carcinoma and p16 immunohistochemistry is statistically significant.

Fischers exact-42.493, degree of freedom=7, p value= <0.001.

| Diagnosis - HPV IHC Cross tabulation | | | | | |
|---|---|---|---|---|---|
| | | | HPV IHC | | Total |
| | | | Negative | Positive | |
| Diagnosis | Keratinizing SCC | Count | 44 | 1 | 45 |
| | | % within Diagnosis | 97.8% | 2.2% | 100.0% |
| | Keratinizing SCC – (b) | Count | 5 | 1 | 6 |
| | | % within Diagnosis | 83.3% | 16.7% | 100.0% |
| | Keratinizing SCC– (a) | Count | 6 | 0 | 6 |
| | | % within Diagnosis | 100.0% | 0.0% | 100.0% |
| | KeratinizingSCC– (c) | Count | 2 | 0 | 2 |
| | | % within Diagnosis | 100.0% | 0.0% | 100.0% |
| | KeratinizingSCC– (d) | Count | 2 | 0 | 2 |
| | | % within Diagnosis | 100.0% | 0.0% | 100.0% |
| | Non Keratinizing SCC | Count | 13 | 20 | 33 |
| | | % within Diagnosis | 39.4% | 60.6% | 100.0% |
| | Non Keratinizing SCC–(f) | Count | 1 | 3 | 4 |
| | | % within Diagnosis | 25.0% | 75.0% | 100.0% |
| | Non keratinizingSCC–(e) | Count | 2 | 0 | 2 |
| | | % within Diagnosis | 100.0% | 0.0% | 100.0% |
| Total | | Count | 75 | 25 | 100 |
| | | % within Diagnosis | 75.0% | 25.0% | 100.0% |

| Chi-Square Tests | | | | |
|---|---|---|---|---|
| | Value | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) |
| Pearson Chi-Square | 44.321[a] | 7 | .000 | .000 |
| Likelihood Ratio | 48.719 | **7** | **.000** | .000 |
| **Fisher's Exact Test** | **42.493** | | | .000 |
| N of Valid Cases | 100 | | | |

a. 12 cells (75.0%) have expected count less than 5. The minimum expected count is .50.

# PHOTOS

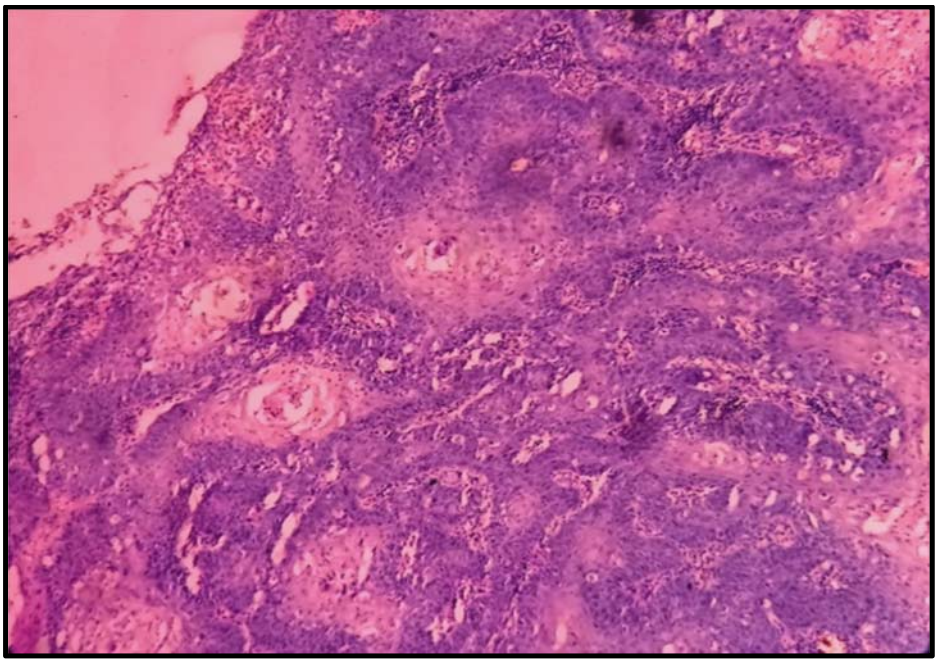


**Fig 1 - Conventional SCC :** Tumor cells show keratinization with keratin pearl formation. H&E 100 X. Case S – 974 /18.

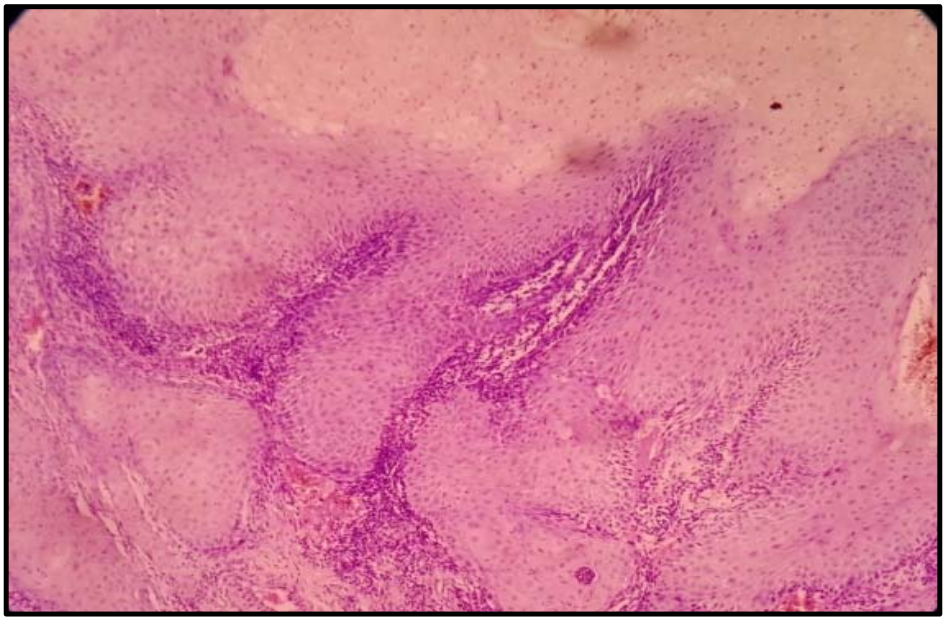


**Fig 2 Verrucous carcinoma:** Tumor cells have pushing bulbous border with well defined cells and less pleomorphism. H&E 100 X. Case S – 611/18.

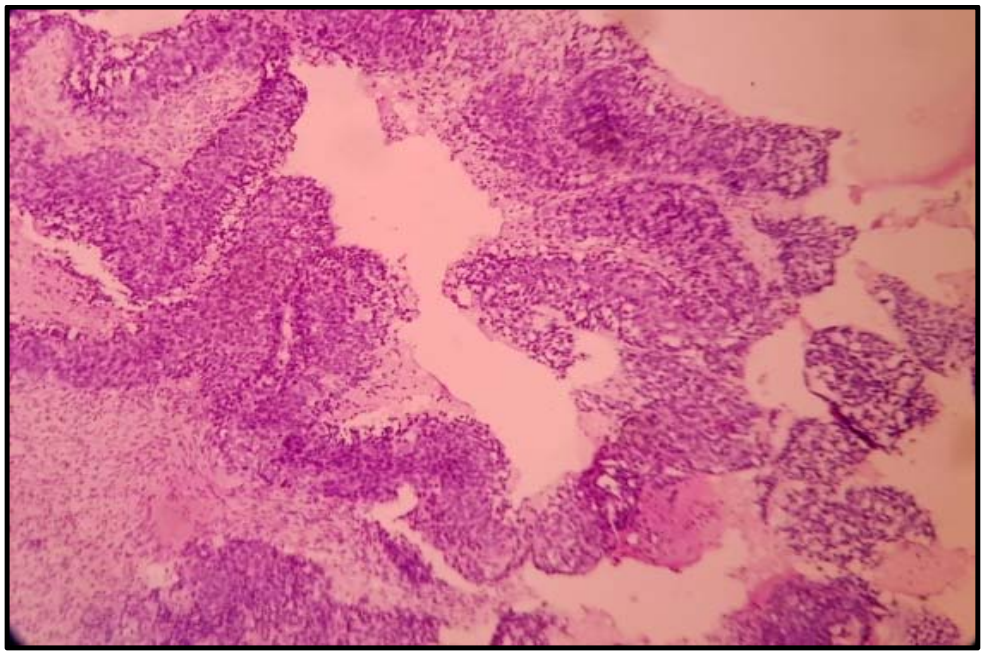


**Fig 3: Papillary variant of SCC:** H&E 100X. Case S – 2007/17

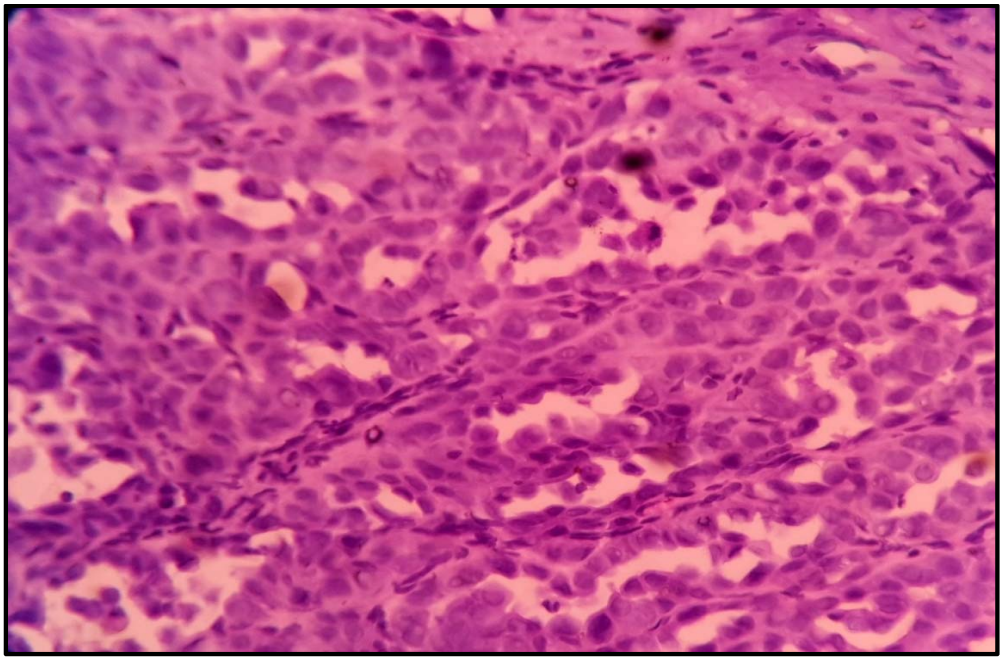


**Fig 4 - Acantholytic variant of SCC:** The tumor cells show acantholysis.

H&E 100 X. Case – S 666 /18.

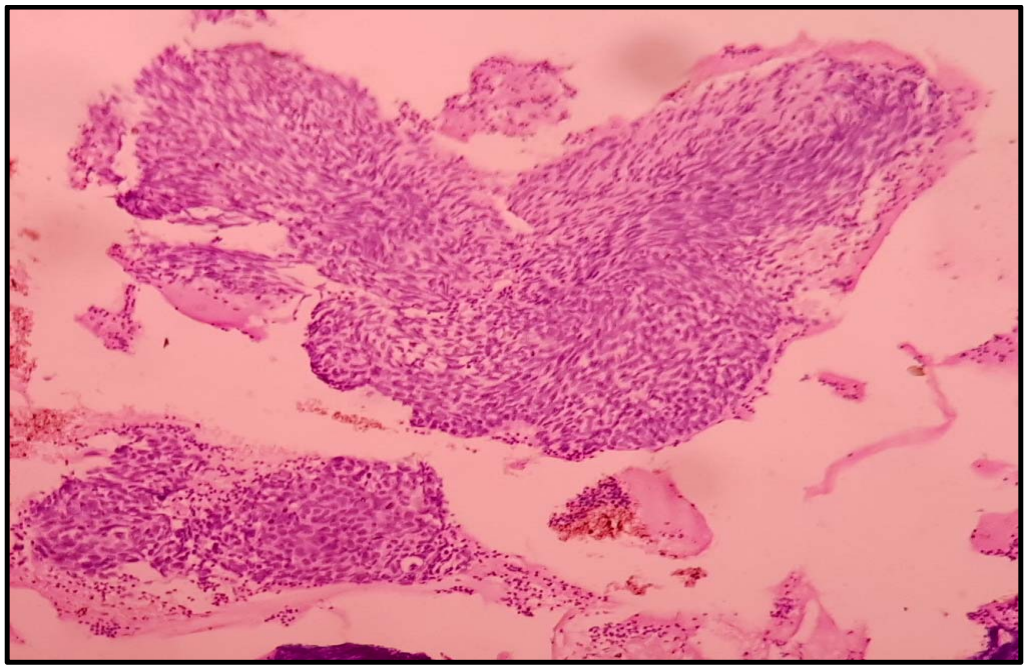


**Fig 5- Sarcomatoid SCC:** Tumor cells are spindle shaped arranged in sheets, admixed with pleomorphic epithelial cells. H&E 100 X. Case S - 1506/17

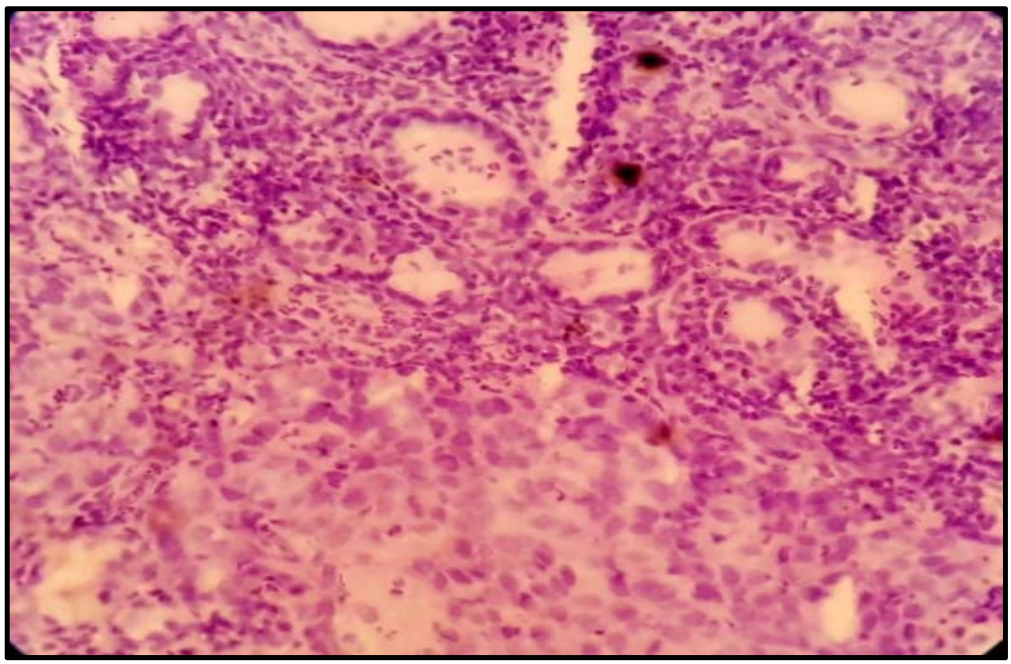


**Fig 6 - Adenosquamus carcinoma:** Tumor show biphasic component of glandular and squamous differentiation. H&E 400 X. Case S – 678/18

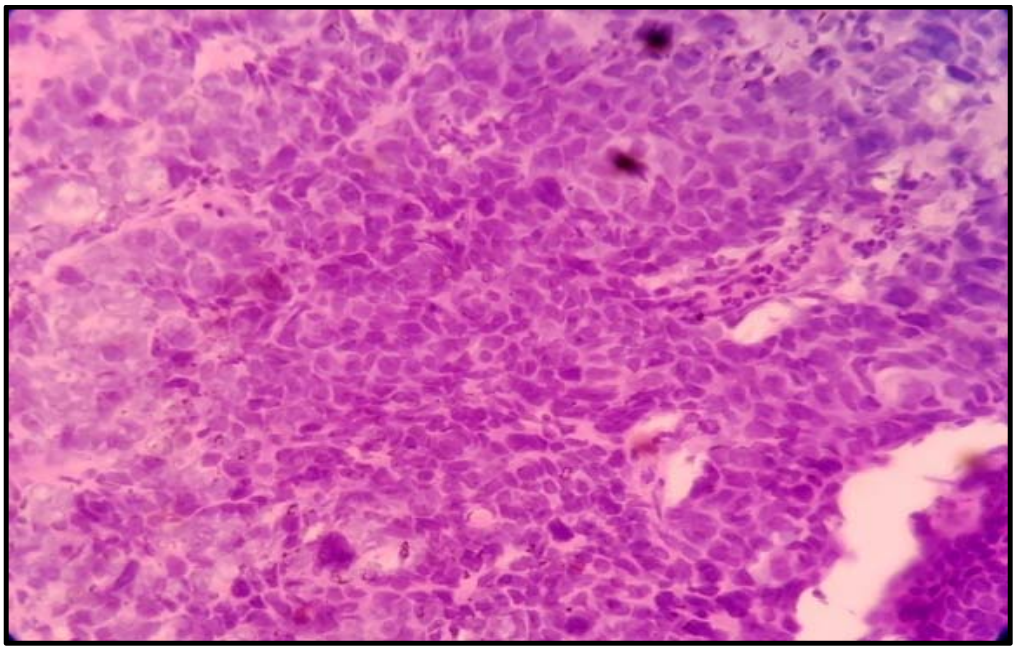


**Fig 7 - Non keratinizing SCC:** The tumorcells are pleomorphic and show no keratinization. H&E 400X. Case S – 1273 /17.

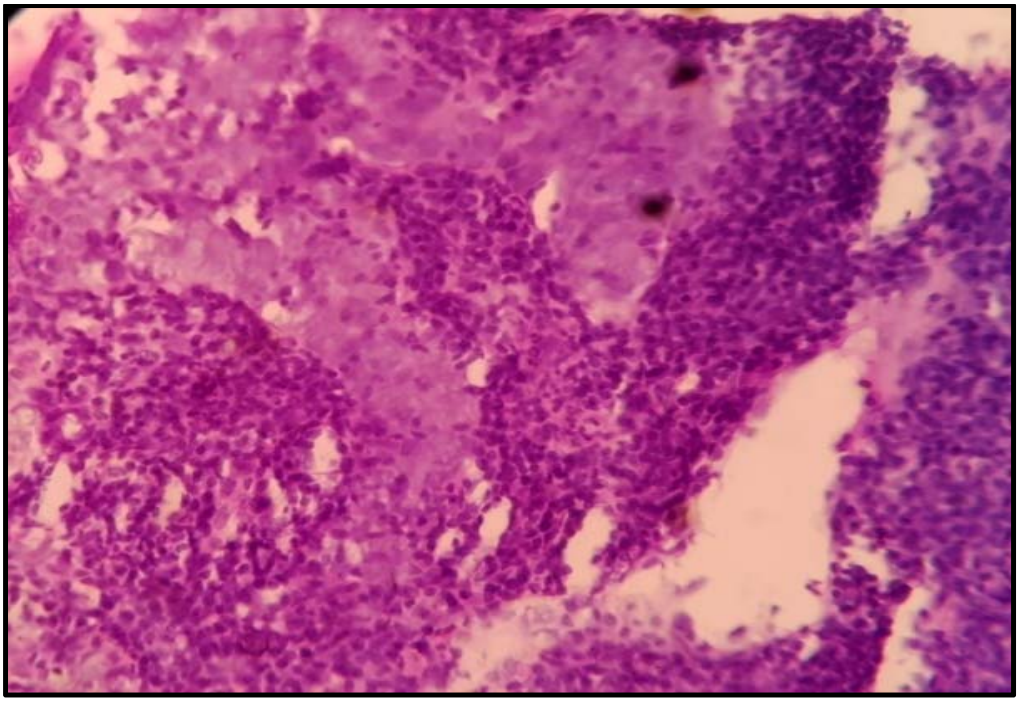


**Fig 8 - Lymphoepithelial carcinoma:** Tumor cells are are poorly differentiated & are admixed with dense lymphocytic infiltrate. H&E 400 X. Case S – 2026 /17.

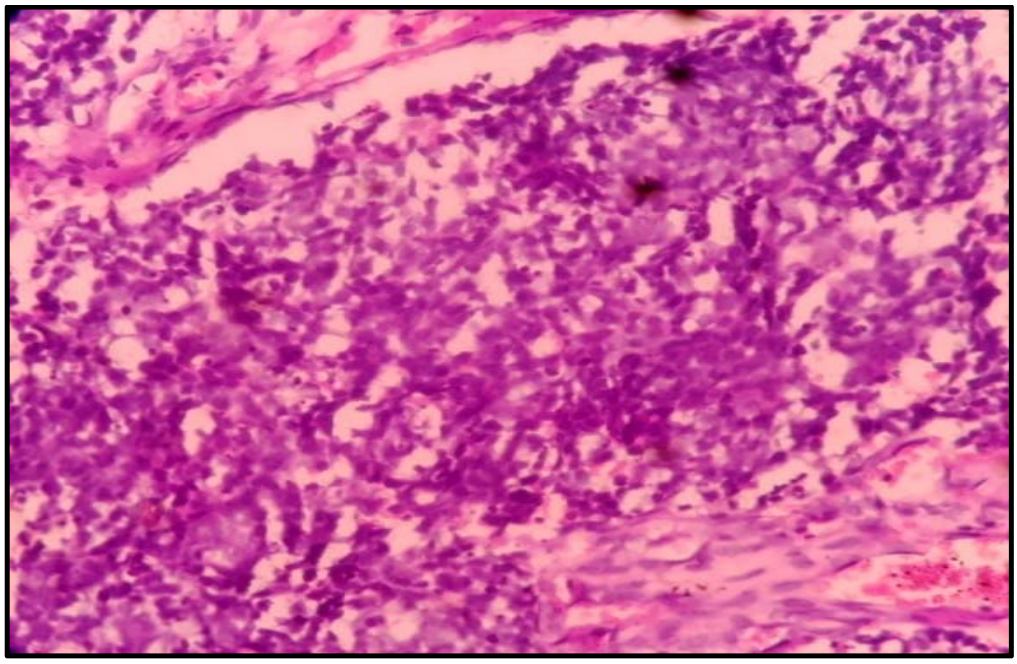


**Fig 9 - Basaloid squamous cell carcinoma:** Tumor cells are basaloid appearing and show less pleomorphism arranges in nests. H&E400 X. Case S – 1560/17.

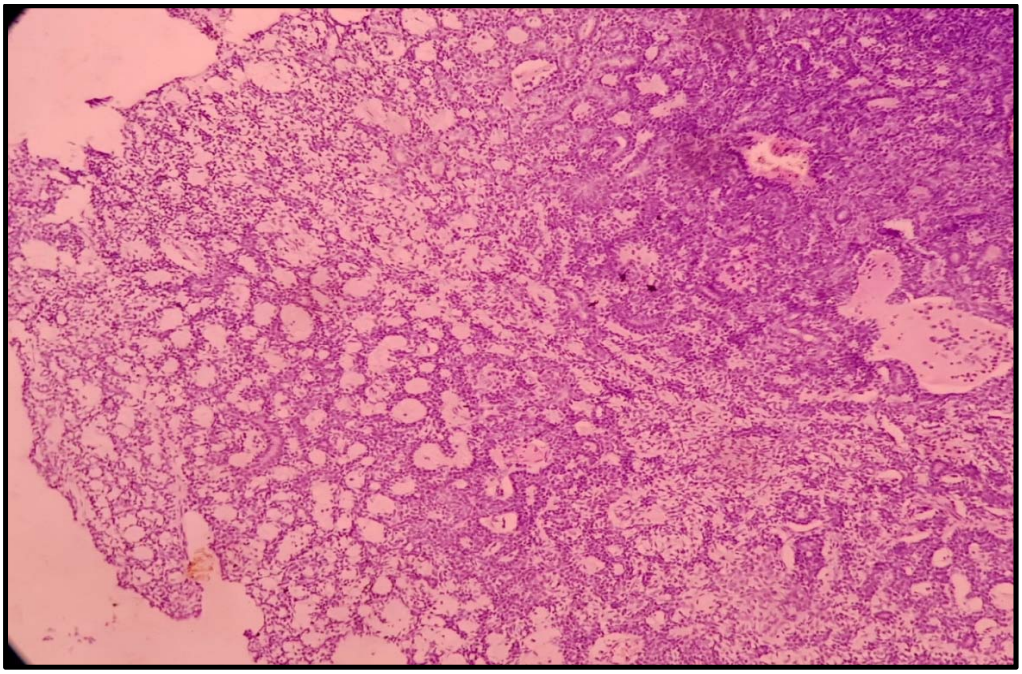


**Fig 10 - Adenoid cystic carcinoma:** Tumor cells are arranged in cribriform and tubular pattern. H&E 100 X. Case S – 938/17

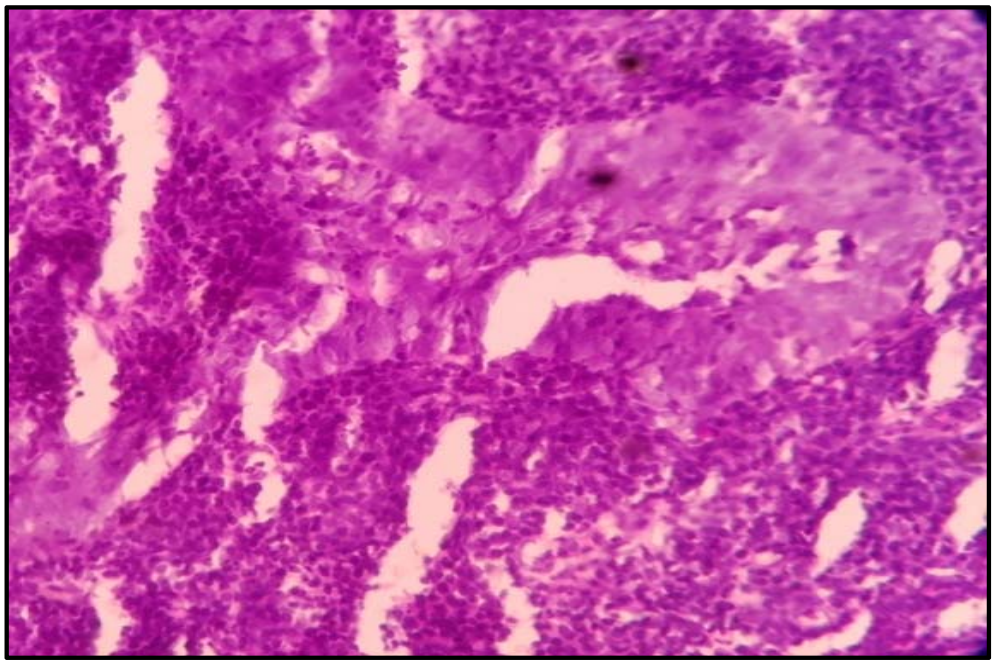


**Fig 11- Nasopharyngeal undifferentiated carcinoma:**Tumor cells arranged in syncytial clusters and admixed with lymphoplasmacytic cells. H&E 400 X. Case S – 1606/17

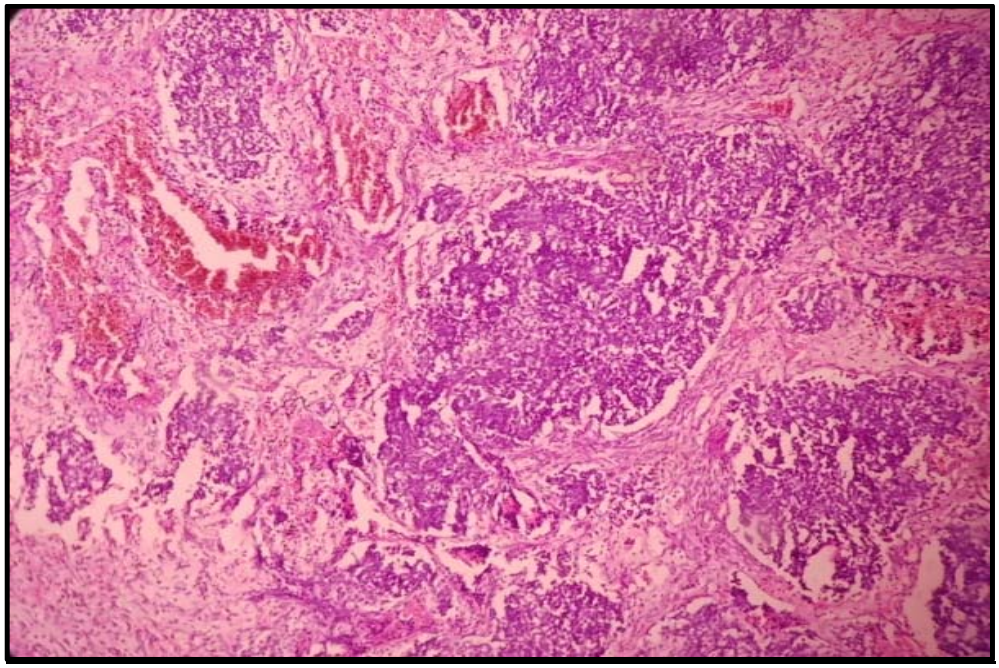


**Fig12 - Sinonasal undifferentiated carcinoma;** H&E 100 X. Case S -794/17

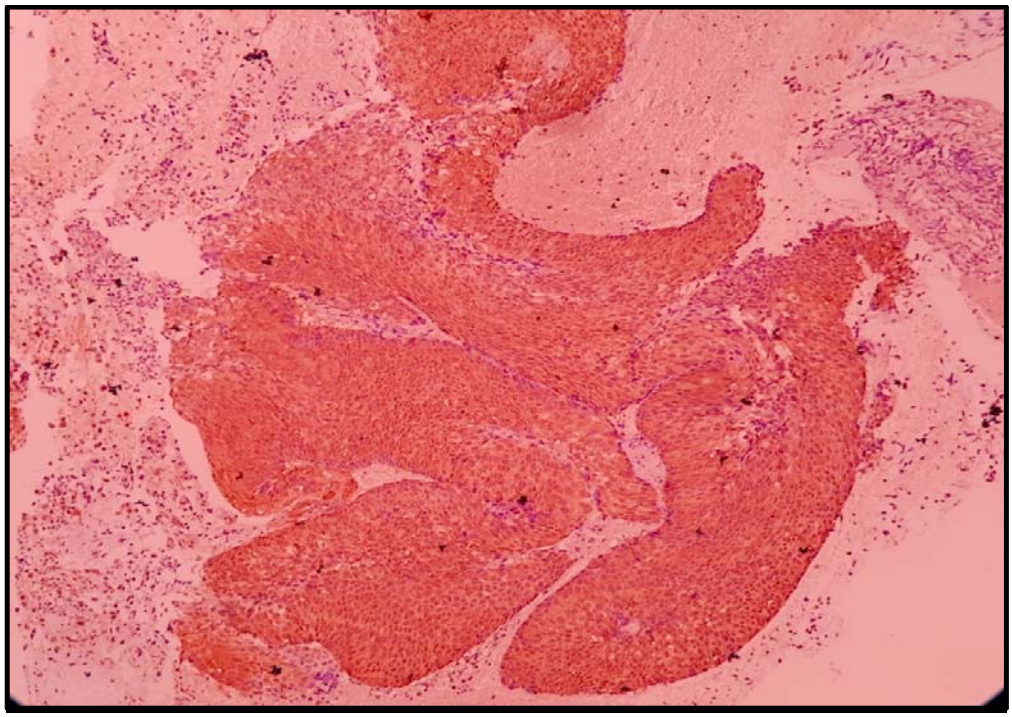


**Fig 13 – p16 positivity:** cells. IHC 100 X. Case S – 1139 /17.

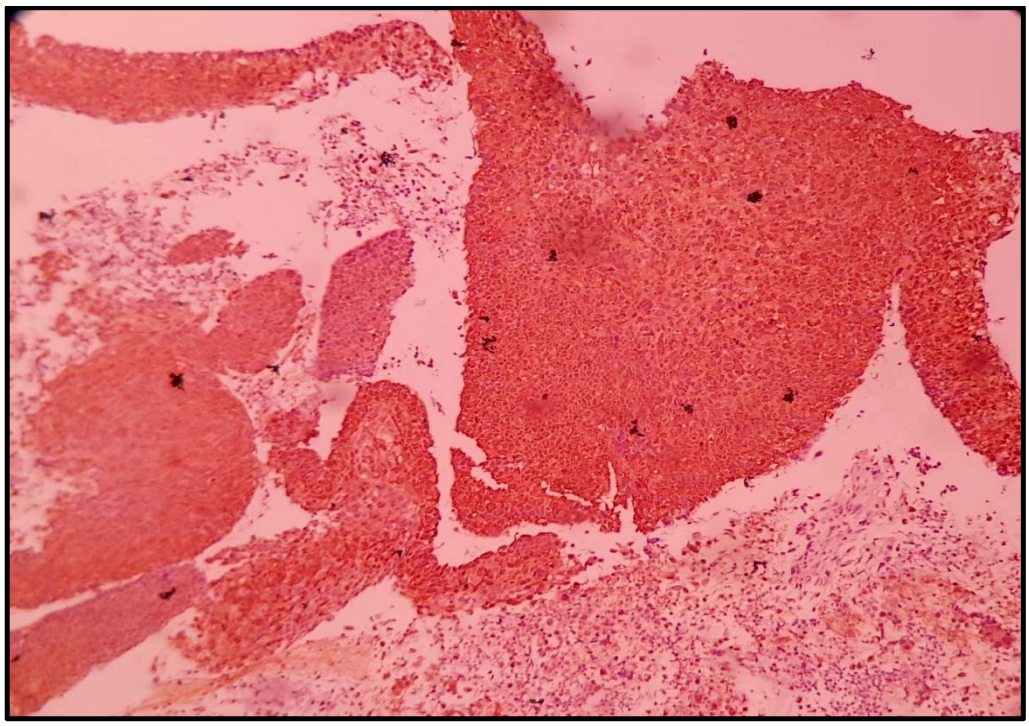


**Fig 14: Diiffuse p 16 positivity :** IHC 100X. Case S – 352/18

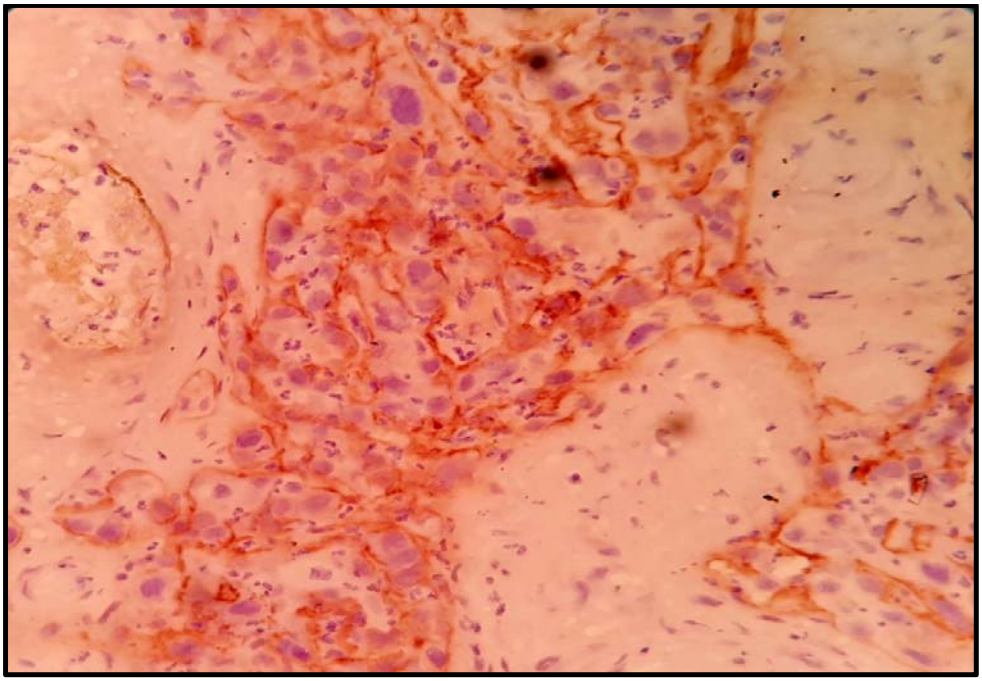


**Fig 15:** p16 negative in keratinizing SCC. IHC 100X. Case S – 428/18

# DISCUSSION

Squamous cell carcinoma is the most common tumor of UADT region & contributes more than 90% of total tumors of UADT. It is usually seen in 5th to 6th decade and has male preponderance. Tobacco, smoking and betel quid are common risk factors with recent increase in incidence of HPV infection. This HPV related SCC is more common in oropharynx than the other sites of UADT. In present study 100 cases of SCC are taken and its age, sex, site wise, histopathological difference and p16 association has been done.

## AGE INCIDENCE

In present study most of cases are seen during 5th decade. In study by sabagesh et al and datta et al, increased incidence was seen during 5thdecade and 4th decade. In table 13 the comparision study is shown.

**Table 13 : Comparision of study of age incidence in conventional SCC**

| S.No | STUDY | AGE INCIDENCE |
|---|---|---|
| 1. | Sabegesh et al | 5th decade |
| 2. | Datta et al | 4th decade |
| 3. | Present study | 5th decade |

In study by Michelle D Williams et al HPV related SCC are commonly seen in less than 50yr of age and in study by ringerstorm HPV related SCC age incidence was lesser than the conventional SCC. Table 14 show the comparision of previous study with present study.

**Table 14 : Comparision of study on HPV related scc age incidence.**

| S.No | STUDY | AGE INCIDENCE |
|---|---|---|
| 1. | Michelle D Williams et al | Less than 50yr |
| 2. | Ringerstorm | Mean age of 45yr |
| 3. | Present study | Mean age of 48yr |

## SEX INCIDENCE

Overall SCC of UADT (whether HPV / Non HPV SCC) is more common in male and in study by Rajesh vaidya incidence in male was 78%, in female 22% and M : F ratio was 3.5 : 1. In study by S. Nair et al incidence was 77% in males, 23% in females and the ratio was 3.3 :1.

**Table 15 : Sex incidence comparision study.**

| S.No. | STUDY | INCIDENCE |
|---|---|---|
| 1. | Rajesh vaidhya | 3.5 : 1 |
| 2. | S. Nair et al | 3.3 : 1 |
| 3. | Present study | 2.8 : 1 |

## ANATOMICAL DISTRIBUTION OF SCC IN UADT

In study by S. Chakkarabarthi, oral cavity is most common site for conventional SCC in UADT constituting upto 62% and in study by MK Gosh et al 35.08% were seen in oral cavity. HPV related SCC commonly affects the oropharynx and in study by El mofty, 68% of the SCC cases were seen in oropharynx. In study by Weinberger et al 61% of HPV related SCC were seen in oropharynx.

**Table 16 : Comparision study of site wise distribution of SCC.**

| S. No | Conventional SCC site wise distribution in UADT study | Incidence | HPV related SCC site wise distribution in UADT study | Incidence |
|---|---|---|---|---|
| 1. | S. Chakkarabarthi – oral cavity | 62% | El mofty - oropharynx | 68% |
| 2. | MK Gosh et al – oral cavity | 35.08% | Weinberger et al - oropharynx | 61% |
| 3. | Present study – oral cavity | 42% | Present study – oropharynx | 56% |

## HISTOLOGICAL CLASSIFICATION OF SCC FOR p16 IHC

In order to study the correlation of HPV in SCC of UADT, they are classified morphologically into keratinizing and non keratinizing SCC, since HPV is seen in non keratinizing variant of SCC. Many studies used morphological system for classification of SCC and they are Wain SL, Kier R et al and Zhang MQ, Davila RM et al studies. It is shown in table 17.

**Table 17 : Comparision of the Study on Morphology for Classification of SCC for p16 IHC**

| S.No | STUDY | METHOD OF CLASSIFICATION |
|---|---|---|
| 1. | Wain SL, Kier R et al | Histopathological method |
| 2. | Zhang MQ, Davila RM et al | Histopathological method |
| 3. | Present study | Histopathological method |

## p16 IN NON KERATINIZING SCC

In present study p 16 IHC is found with higher percentage in non keratinizing SCC (59%) than the keratinizing SCC (3%) and anatomical site wise they were seen mostly in oropharyngeal and in some hypopharyngeal areas. Similar picture of p16 IHC and site were seen in study by Licitra et al and Reimers et al. It is shown in the following table18.

**Table 18 : Comparision Of Studies of p16 In Non Keratinizing SCC**

| S.No. | STUDY | INCIDENCE |
|---|---|---|
| 1. | Licitra et al | 89% |
| 2. | Reimers et al | 92% |
| 3. | Present study | 59% |

## SUMMARY

In the present prospective study, 100 cases of squamous cell carcinoma of upper aerodigestive tract were correlated with histopathological findings and immunohistochemistry was done in these 100 cases to find the correlation of HPV related carcinoma in UADT. The following results were obtained.

Of the 3750 small biopsy specimens received in our department during july 2016 to july 2018 study period. 850 cases were from upper aerodigestive tract with an incidence of 23%.

In 850 cases 248 (32%) cases were benign and 527 (68%) cases were malignant and 75 cases were inadequate for reporting.

In 527 malignant tumors of upper aerodigestive tract, 496 cases were of squamous cell carcinoma with an incidence of 94%. The salivary gland tumors were second common tumor with total cases of 13 and an incidence of 2.5% . Least common were the malignant lymphomas and melanomas.

In 496 cases of squamous cell carcinomas, oral cavity showed increased incidence of 42% and the total number of cases were 212. The second common site was oropharynx with 120 cases and an incidence of 24%. The least common site for SCC was sinonasal region with an incidence of 2%

From 496 cases, 100 cases were taken and they were histologically studied and classified as keratinizing and non keratinizing SCC. It showed 61 cases showed keratinzing morphology and 39 cases with non keratinizing morphology.

There was an increase in the incidence of cases with keratinizing SCC variant in the oral cavity compared to the laryngeal region (65.5% and 13% respectively).

Non keratinizing SCC variant was seen more in oropharynx than in hypopharynx (72% and 10% respectively).

Overall there was male predominance(74%) in our study. Incidence among females was 26%

Mean age of incidence for non keratinizing SCC was 48 years and keratinizing SCC was 58 years.

p16 immunohistochemistry was done in these 100 cases and positivity was seen more in non keratinizing SCC(59%) with the p value of 0.001. Hence HPV has strong correlation with non keratinizing morphological variant of SCC and p16 can be used for determination of HPV status.

# CONCLUSION

With emergence of HPV positive upper aerodigestive tract squamous cell carcinoma, as an unique type, it is important to identify such patients by testing HPV in routine clinical practice. This can be carried by various methods. In present study we have used p16 as a surrogate marker for testing HPV and cases were selected taking into account its histopathological feature. Since squamous cell carcinoma with non keratinizing morphology show increased positive for p16 than the keratinizing squamous cell carcinoma, this feature can be taken as a clue for HPV infection. This shows that light microscopic examination plays an integral part in detection of HPV related carcinomas. This knowledge of HPV infection is important as it carries both patient prognosis and for establishment of specific treatments.

## ANNEXURE – I

## WHO CLASSIFICATION OF EPITHELIAL TUMORS OF SINONASAL REGION:

**Carcinomas**

Keratinizing squamous

cell carcinoma

Non-keratinizing squamous cell carcinoma

Spindle cell squamous cell carcinoma

Lymphoepithelial carcinoma

Sinonasal undifferentiated carcinoma

NUT carcinoma

Neuroendocrine carcinomas

Small cell neuroendocrine carcinoma

Large cell neuroendocrine carcinoma

Adenocarcinomas

lntestinal-type adenocarcinoma

Non-intestinal-type adenocarcinoma

**Teratocarcinosarcoma**

**Sinonasal papillomas**

Sinonasal papilloma, inverted type

Sinonasal papilloma, oncocytic type

Sinonasal papilloma, exophytic type

**Respiratory epithelial lesions**

Respiratory epithelial adenomatoid hamartoma

Seromucinous hamartoma

**Salivary gland tumours**

Pleomorphic adenoma

## WHO CLASSIFICATION OF EPITHELIAL TUMORS OF NASOPHARYNX:

**Carcinomas**

Nasopharyngeal carcinoma

Non-keratinizing squamous cell carcinoma

Keratinizing squamous cell carcinoma

Basaloid squamous cell carcinoma

Nasopharyngeal papillary adenocarcinoma

**Salivary gland tumours**

Adenoid cystic carcinoma

Salivary gland anlage tumour

**Benign and borderline lesions**

Hairy polyp

Ectopic pituitary adenoma

Craniopharyngioma

## WHO CLASSIFICATION OF EPITHELIAL TUMORS OF ORAL CAVITY:

**Malignant Epithelial tumours**

Squamous cell carcinoma

Oral epithelial dysplasia

- Low grade
- High grade

Proliferative verrucous leukoplakia

**Oral mucosal melanoma**

**Benign Epithelial tumors**

Squamous cell papilloma

Condyloma acuminatum

Verruca vulgaris

Multifocal epithelial hyperplasia

**Salivary type tumours**

Mucoepidermoid carcinoma

Pleomorphic adenoma

## WHO CLASSIFICATION OF EPITHELIAL TUMORS OF ORAL CAVITY:

**Squamous cell carcinoma**

Squamous cell carcinoma, HPV-positive

Squamous cell carcinoma, HPV-negative

**Salivary gland tumours**

Pleomorphic adenoma

Adenoid cystic carcinoma

Polymorphous adenocarcinoma

## WHO CLASSIFICATION OF EPITHELIAL TUMORS OF LARYNX AND HYPOPHARYNX:

**Malignant surface epithelial tumours**

Conventional squamous cell carcinoma

Verrucous squamous cell carcinoma

Basaloid squamous cell carcinoma

Papillary squamous cell carcinoma

Spindle cell squamous cell carcinoma

Adenosquamous carcinoma

Lymphoepithelial carcinoma

**Precursor lesions**

Dysplasia, low grade

Dysplasia, high grade

Squamous cell papilloma

Squamous cell papillomatosis

**Neuroendocrine tumours**

Well-differentiated neuroendocrine carcinoma

Moderately differentiated neuroendocrine carcinoma

Poorly differentiated neuroendocrine carcinoma

Small cell neuroendocrine carcinoma

Large cell neuroendocrine carcinoma

**Salivary gland tumours**

Adenoid cystic carcinoma

Pleomorphic adenoma

Oncocytic papillary cystadenoma

## ANNEXURE II

## TNM staging of carcinoma of UADT

**T** - Primary tumour

**TX**- Primary tumour cannot be assessed

**TO** - No evidence of primary tumour

**Tis** - Carcinoma in situ

**Sino-nasal region:**

**Maxillary sinus region**

**T1** – Tumour is limited to the antral mucosa. with no erosion or with destruction of the bone

**T2** - Tumour causing bone erosion or destruction and including the extension into hard palate and or middle nasal meatus, except extension to the posterior wall of maxillary sinus and pterygoid plate.

**T3** - Tumour invades any of the following: posterior wall of maxillary sinus, subcutaneous tissues, floor or medial wall of the orbit, pterygoid fossa and ethmoid sinuses

**T4a** - Tumour invades any of the following: skin of cheeks, anterior orbital contents, cribriform plate, pterygoid plates, infratemporal fossa, sphenoid or frontal sinuses

**T4b** - Tumour invades any of the following: orbital apex, nasopharynx, clivus, dura, brain, middle cranial fossa, cranial nerves other than maxillary division of trigeminal nerve.

**Nasal cavity and ethmoid sinus region**

**T1** – Tumour is limited to one subsite of nasal cavity / ethmoid sinus, with / without bony invasion

**T2** - Tumour involves two subsites in a single site / extends to involve an adjacent site within the nasoethmoidal complex, with or without bony invasion

**T3** - Tumour extends to invade the medial wall / floor of the

Orbit/ maxillary sinus / palate / cribriform plate

**T4a , T4b** – same as in maxillary sinus

**Nasopharyngeal region:**

**T1** – Tumour is confined to the nasopharynx or extends to oropharynx and or nasal cavity

**T2** - Tumour with parapharyngeal extension (i.e. posterolateral infiltration of the tumour)

**T3** - Tumour has invaded the bony structures of skull base and or paranasal sinuses

**T4** - Tumour with intracranial extension and or involvement of the cranial nerves / infratemporal fossa / hypopharynx / orbit / masticator space

**Lip, oral cavity and oropharynx carcinoma :**

**T1** - Tumour less than 2 cm in greatest dimension

**T2** - Tumour more than 2 cm but less than 4 cm in greatest dimension

**T3** - Tumour  more than 4 cm in greatest dimension

**T4a - (lip)**

Tumour invaded through the cortical bone, inferior alveolar nerve and floor of mouth / skin (nose or chin)

**T4a (oral cavity)**

Tumour invaded through the cortical bone and into deep/extrinsic muscle of tongue (hyoglossus, genioglossus, styloglossus and palatoglossus )maxillary sinus / skin of face

**T4b (lip and oral cavity)**

Tumour invaded the masticator space / pterygoid plates / skull base / encases the internal carotid artery.

**Larynx**:

**Supraglottis**

**T1** – Tumour is limited to one subsite of supraglottis, with vocal cord mobility

**T2** – Tumour has invaded the mucosa of more than one adjacent subsite of supraglottis / glottis / region outside the supraglottis (e.g. base of tongue, vallecula / medial wall of pyriform sinus), without fixation of the larynx

**T3** – Tumour is limited to larynx with fixation of vocal cord and/or invades any of the following: pre- epiglottic s[ace, postcricoid area, paraglottic space, inner aspect of thyroid cartilage

**T4a** - Tumour invades through thyroid cartilage and or invades tissues beyond the larynx; example: trachea, soft tissues of neck, muscle of tongue, strap muscles, thyroid and oesophagus

**T4b** - Tumour has invaded the prevertebral space / mediastinal structures / encases the carotid artery.

**Glottis**

**T1** - Tumour limited to one or both vocal cords (may involve anterior or posterior commissure), with vocal cord mobility

**T1a** - Tumour limited to one vocal cord

**T1b** - Tumour involves both the vocal cords

**T2** - Tumour extends to supraglottis and or subglottis, and or with impaired mobility of vocal cord.

**T3** - Tumour limited to larynx with fixation of vocal cord and or invades paraglottic space and or inner aspect of the thyroid cartilage

**T4a, T4b** – same as supraglottic tumor.

**Subglottis region**

**T1** - Tumour is limited to the subglottis

**T2** - Tumour extends to one or both vocal cords, with normal / impaired Mobility

**T3** - Tumor is limited to larynx with fixation of the vocal cord.

**T4a, T4b** – Same as supraglottic tumor**.**

**Hypopharynx region:**

**T1** - Tumour is limited to one subsite of hypopharynx and or less than 2 cm in greatest dimension

**T2** - Tumour invaded more than one subsite of hypopharynx or an adjacent site / measuring more than 2 cm but less than 4 cm in greatest dimension, without fixation of hemilarynx

**T3** - Tumour more than 4 cm in greatest dimension / with fixation of hemilarynx / extension to esophagus

**T4a** - Tumour invaded any of the following: thyroid/cricoids cartilage, hyoid bone, thyroid gland, esophagus and central compartment soft tissue

**T4b** - Tumour invades prevertebral fascia, encases carotid

Artery / invades mediastinal structures.

**N - Regional lymph nodes ( cervical nodes)**

**NX** - Regional lymph nodes cannot be assessed

**NO** - No regional lymph node metastasis

**N1** - Metastasis in a single ipsilateral lymph node, less than 3 cm in greatest dimension

**N2** - Metastasis as specified in N2a/ N2b/ N2c

**N2a** - Metastasis in a single ipsilateral lymph node, more than 3 cm but less than 6 cm in greatest dimension

**N2b** - Metastasis in multiple ipsilateral lymph nodes, all less than 6 cm in greatest dimension

**N2c** - Metastasis in bilateral or contralateral lymph nodes, all less than 6 cm in greatest dimension

**N3** - Metastasis in a lymph node more than 6 cm in greatest dimension

**M - Distant metastasis**

**MO** - No distant metastasis

**M1** - Distant metastasis

**Stage grouping**

| | | | |
|---|---|---|---|
| Stage 0 | Tis | N0 | M0 |
| Stage 1 | T1 | N0 | M0 |
| Stage 2 | T2 | N0 | M0 |
| Stage 3 | T1-2 | N1 | M0 |
| | T3 | N0-1 | M0 |
| Stage IVA | T1-3 | N2 | M0 |
| | T4a | N0-2 | M0 |
| Stage IVB | T4b | Any N | M0 |
| | AnyT | N3 | M0 |
| Stage IVC | AnyT | Any N | M1 |

## ANNEXURE III

## PROFORMA

Name: Age / Sex:

IP No: Unit & Ward:

HPE No:

H/O Presenting illness:

Significant past history (if any):

Type of specimen:

Anatomical site:

Laterality:

Details of any relevant imaging studies:

Details of any investigation for metastatic carcinoma:

**MICROSCOPIC FINDINGS**

Histological type

Histological grade

Mitosis

Lymphovascular space invasion

Perineural invasion

Additional pathological findings

Immunohistochemistry

## ANNEXURE: IV

## HAEMATOXYLIN AND EOSIN STAINING METHOD FOR HISTOPATHOLOGY.

1.Sections will be dewaxed with xylene for 20 minutes.

2.Sections will be hydrated through descending concentrations (absolute alcohol, 90%, 70%, 50%) of ethanol to water solutions.

3.Sections will be rinsed in distilled water.

4.Sections will be placed in Ehrlich haematoxylin stain for 20-30minutes.

5.Sections will be rinsed with water.

6.Differentiation will be done by immersing the sections in 1% acid alcohol for 10 seconds.

7.Sections will be rinsed with water.

8.Blueing will be done by keeping the sections in scott's tap water for 2-10 minutes.

9.Counterstaining will be done with 1% aqueous eosin for 1-3 minutes.

10.Sections will be rinsed with water.

11.Sections will be dehydrated through increasing concentration of ethanol solutions (50%,70%, 95%, absolute alcohol) and cleared with xylene.

12.Sections will be mounted with DPX.

## ANNEXURE: V

## KEY TO MASTER CHART

### SITE OF INVOLVEMENT

**1-** Oral cavity

**2-** Oropharynx

**3-** Sinonasal

**4-** Nasopharynx

**5-** Hypopharynx

**6-** Larynx

### VARIANTS OF SCC:

a – Verrucous carcinoma

b – Papillary carcinoma

c – Acantholytic carcinoma

d – Sarcomatoid carcinoma

e – Lymphoepithelial carcinoma

f – Basosquamous carcinoma

M – Male

F – Female

# ANNEXURE VI

# MASTER CHART

| Serial no. | Age | Sex | Site | Biopsy no. | Diagnosis | HPV IHC |
|---|---|---|---|---|---|---|
| 1. | 46 | M | 6 | S – 1508/17 | Keratinizing SCC | Negative |
| 2. | 36 | F | 2 | S - 1528/16 | Non Keratinizing SCC | Positive |
| 3. | 37 | M | 1 | S – 1541/16 | Keratinizing SCC- (a) | Negative |
| 4. | 45 | M | 6 | S – 1573/16 | Keratinizing SCC | Negative |
| 5. | 50 | M | 2 | S – 1751/16 | Non Keratinizing SCC | Positive |
| 6. | 70 | M | 1 | S – 1769/16 | Non Keratinizing SCC | Negative |
| 7. | 60 | M | 1 | S – 1815/16 | Keratinizing SCC– (b) | Negative |
| 8. | 62 | F | 6 | S – 1889/16 | Non Keratinizing SCC | Negative |
| 9. | 55 | F | 2 | S – 1943/16 | Non Keratinizing SCC | Negative |
| 10. | 60 | M | 1 | S – 2011/16 | Keratinizing SCC | Negative |
| 11. | 50 | M | 1 | S – 2047/16 | Non KeratinizingSCC (f) | Negative |
| 12. | 45 | F | 5 | S - 2153/16 | Keratinizing SCC – (b) | Negative |
| 13. | 70 | M | 1 | S – 2120/16 | Keratinizing SCC | Negative |
| 14. | 58 | M | 1 | S – 2256/16 | Keratinizing SCC(a) | Negative |
| 15. | 56 | M | 1 | S – 2266/16 | Keratinizing SCC | Negative |
| 16. | 50 | F | 2 | S – 80/17 | Non Keratinizing SCC | Positive |
| 17. | 31 | M | 2 | S – 231/17 | Non Keratinizing SCC | Positive |
| 18. | 74 | M | 1 | S – 459/17 | Keratinizing SCC | Negative |
| 19. | 61 | M | 1 | S – 467/17 | Keratinizing SCC | Negative |
| 20. | 73 | M | 6 | S – 535/17 | Keratinizing SCC | Negative |
| 21. | 60 | M | 1 | S – 548/17 | Keratinizing SCC | Negative |
| 22. | 53 | M | 2 | S – 689/17 | Non Keratinizing SCC | Positive |
| 23. | 64 | M | 2 | S – 698/17 | Non Keratinizing SCC | Positive |

| | | | | | | |
|---|---|---|---|---|---|---|
| 24. | 50 | M | 2 | S – 703/17 | Non Keratinizing SCC | Negative |
| 25. | 62 | M | 1 | S – 707/17 | Keratinizing SCC | Negative |
| 26. | 69 | M | 1 | S – 748/17 | Keratinizing SCC | Negative |
| 27. | 65 | M | 1 | S – 770/17 | Keratinizing SCC | Negative |
| 28. | 65 | M | 2 | S – 817/17 | KeratinizingSCC– (b) | Negative |
| 29. | 40 | M | 2 | S – 827/17 | Non Keratinizing SCC | Positive |
| 30. | 60 | M | 6 | S – 854/17 | Non Keratinizing SCC | Negative |
| 31. | 55 | M | 1 | S – 893/17 | Keratinizing SCC | Negative |
| 32. | 47 | M | 2 | S – 901/17 | Non Keratinizing SCC | Positive |
| 33. | 62 | M | 1 | S – 962/17 | Keratinizing SCC | Negative |
| 34. | 65 | M | 1 | S – 963/17 | Keratinizing SCC | Negative |
| 35. | 63 | M | 2 | S – 1030/17 | Keratinizing SCC | Negative |
| 36. | 70 | M | 1 | S - 1095 /17 | Keratinizing SCC | Negative |
| 37. | 58 | M | 6 | S – 1100/17 | Keratinizing SCC- (b) | Positive |
| 38. | 32 | M | 2 | S – 1105/17 | Non keratinizing SCC | Positive |
| 39. | 31 | F | 2 | S – 1139/17 | Non Keratinizing SCC | Positive |
| 40. | 70 | F | 5 | S – 1176/17 | Non Keratinizing SCC | Negative |
| 41. | 36 | M | 2 | S – 1250/17 | Non Keratinizing SCC | Positive |
| 42. | 55 | F | 3 | S – 1261/17 | Keratinizing SCC | Negative |
| 43. | 52 | M | 4 | S – 1262/17 | Keratinizing SCC | Negative |
| 44. | 60 | M | 4 | S – 1269/17 | Keratinizing SCC | Negative |
| 45. | 51 | M | 2 | S – 1273/17 | Non Keratinizing SCC | Negative |
| 46. | 68 | M | 2 | S – 1313/17 | KeratinizingSCC– (c) | Negative |
| 47. | 62 | M | 2 | S – 1428/17 | Non keratinizing SCC | Negative |
| 48. | 62 | M | 6 | S – 1506/17 | KeratinizingSCC– (d) | Negative |
| 49. | 55 | M | 1 | S – 1528/17 | Keratinizing SCC | Negative |
| 50. | 50 | F | 2 | S – 1560/17 | Non Keratinizing SCC(f) | Positive |
| 51. | 35 | F | 5 | S – 1627/17 | Keratinizing SCC | Negative |
| 52. | 24 | F | 5 | S – 1702/17 | Keratinizing SCC | Negative |

| | | | | | | |
|---|---|---|---|---|---|---|
| 53. | 59 | M | 1 | S – 1828/17 | Keratinizing SCC | Negative |
| 54. | 52 | M | 2 | S – 1853/17 | Non Keratinizing SCC | Negative |
| 55. | 50 | F | 5 | S – 1874/17 | Non keratinizingSCC–(f) | Positive |
| 56. | 64 | M | 2 | S – 1930/17 | Keratinizing SCC | Positive |
| 57. | 50 | M | 6 | S – 2007/17 | KeratinizingSCC– (b) | Negative |
| 58. | 45 | F | 3 | S – 2026/17 | Non keratinizingSCC–(e) | Negative |
| 59. | 65 | M | 1 | S – 2034/17 | Keratinizing SCC | Negative |
| 60. | 45 | M | 1 | S – 2074/17 | Keratinizing SCC | Negative |
| 61. | 52 | M | 2 | S – 2078/17 | Non KeratinizingSCC–(f) | Positive |
| 62. | 62 | M | 2 | S – 2091/17 | Non Keratinizing SCC | Negative |
| 63. | 77 | M | 3 | S – 2154/17 | Keratinizing SCC | Negative |
| 64. | 65 | F | 4 | S – 2170/17 | Keratinizing SCC | Negative |
| 65. | 54 | M | 3 | S – 8/18 | Keratinizing SCC | Negative |
| 66. | 18 | F | 5 | S – 106/18 | Non Keratinizing SCC | Negative |
| 67.. | 60 | M | 6 | S – 175/18 | KeratinizingSCC– (d) | Negative |
| 68. | 55 | M | 2 | S – 352/18 | Non Keratinizing SCC | Positive |
| 69. | 58 | M | 1 | S – 428/18 | Keratinizing SCC | Negative |
| 70. | 45 | F | 4 | S – 464/18 | Non keratinizingSCC– (e) | Negative |
| 71. | 55 | F | 1 | S – 538/18 | Keratinizing SCC | Negative |
| 72. | 49 | M | 1 | S – 582/18 | Keratinizing SCC | Negative |
| 73. | 75 | F | 1 | S – 611/18 | Keratinizing SCC(a) | Negative |
| 74. | 65 | M | 4 | S – 656/18 | Non Keratinizing SCC | Negative |
| 75. | 65 | M | 1 | S – 666/18 | KeratinizingSCC– (c) | Negative |
| 76. | 70 | F | 1 | S – 695/18 | Keratinizing SCC– (a) | Negative |
| 77. | 63 | M | 1 | S – 700/18 | Keratinizing SCC | Negative |
| 78. | 36 | M | 2 | S – 751/18 | Non keratinizing SCC | Positive |
| 79. | 49 | M | 1 | S – 760/18 | Keratinizing SCC | Negative |
| 80. | 47 | M | 2 | S – 764/18 | Non Keratinizing SCC | Positive |

| | | | | | | |
|---|---|---|---|---|---|---|
| 81. | 40 | F | 1 | S – 779/18 | Keratinizing SCC | Negative |
| 82. | 50 | M | 2 | S – 848/18 | Non Keratinizing SCC | Positive |
| 83. | 50 | F | 1 | S – 913/18 | Keratinizing SCC | Negative |
| 84. | 60 | M | 1 | S 929/18 | Keratinizing SCC | Negative |
| 85. | 52 | M | 2 | S – 930/18 | Non keratinizing SCC | Positive |
| 86. | 56 | M | 1 | S – 974/18 | Keratinizing SCC | Negative |
| 87. | 69 | M | 1 | S – 987/18 | Keratinizing SCC | Negative |
| 88. | 40 | M | 2 | S – 995/18 | Non keratinizing SCC | Positive |
| 89. | 39 | M | 2 | S – 1046/18 | Non Keratinizing SCC | Positive |
| 90. | 56 | F | 1 | S – 1048/18 | Keratinizing SCC | Negative |
| 91. | 51 | M | 1 | S – 1112/18 | Keratinizing SCC | Negative |
| 92. | 51 | F | 1 | S – 1136/18 | Keratinizing SCC | Negative |
| 93. | 54 | F | 6 | S – 1146/18 | Keratnizing SCC– (b) | Negative |
| 94. | 65 | M | 1 | S – 1265/18 | Keratinizing SCC- (a) | Negative |
| 95. | 60 | M | 1 | S – 1276/18 | Keratinizing SCC | Negative |
| 96. | 47 | M | 1 | S -1407/18 | Keratinizing SCC | Negative |
| 97. | 40 | F | 2 | S – 1563/18 | Non Keratinizing SCC | Positive |
| 98. | 47 | M | 5 | S – 1662/18 | Non keratinizing SCC | Positive |
| 99. | 38 | M | 2 | S – 1674/18 | Non Keratinizing SCC | Negative |
| 100. | 62 | F | 1 | S – 1896/18 | Keratinizing SCC(a) | Negative |

## ANNEXURE – VII

## LIST OF ABBREVATIONS

AJCC – American Joint Committee on Cancer

CD – Cluster of Differentiation

CEA – Carcino Embryonic Antigen

CK – Cyto Keratin

CTNNBI – Catenin beta 1

DOG – Discovered On GIST

EBV – Epstein Barr Virus

EBER – Epstein Barr virus coded small Ribonucleic acid

EMA – Epithelial Membrane Antigen

FIGO – Federation of Gynecology and Obstetrics

HPV – Human Papilloma Virus

HPF – High Power Field

LMW CK – Low Molecular Weight Cyto Keratin.

NF- kβ – Nuclear Factor kappa light chain enhancer of activated B cells.

NSE – Neuron Specific Enolase

NUT – NUclear protein in Testis

PAS – Periodic Acid Schiff

SCC – Squamous Cell Carcinoma

TTF1 – Thyroid Transcription Factor 1

UADT – Upper Aero Digestive Tract

UICC – Union for International Cancer Control

VEGF – Vascular endothelial growth factor

WHO – World Health Organization

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## ANNEXURE – VIII

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# ANNEXURE – IX

## INSTITUTE OF ETHICAL COMMITTEE APPROVAL


**MADURAI MEDICAL COLLEGE**
**MADURAI, TAMILNADU, INDIA -625 020**
(Affiliated to The Tamilnadu Dr.MGR Medical University, Chennai, Tamil Nadu)




### *ETHICS COMMITTEE CERTIFICATE*

| | | |
|---|---|---|
| Name of the Candidate | : | Dr.M.Meenambigai |
| Course | : | PG in MD., Pathology |
| Period of Study | : | 2016-2019 |
| College | : | MADURAI MEDICAL COLLEGE |
| Research Topic | : | Histopathological analysis and expression of p16 in squamous cell carcinoma of upper-aerpdogestove tract byimmunohistochemistry |
| Ethical Committee as on | : | 13.04.18 |

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

Member Secretary | Chairman | Dean / Convenor

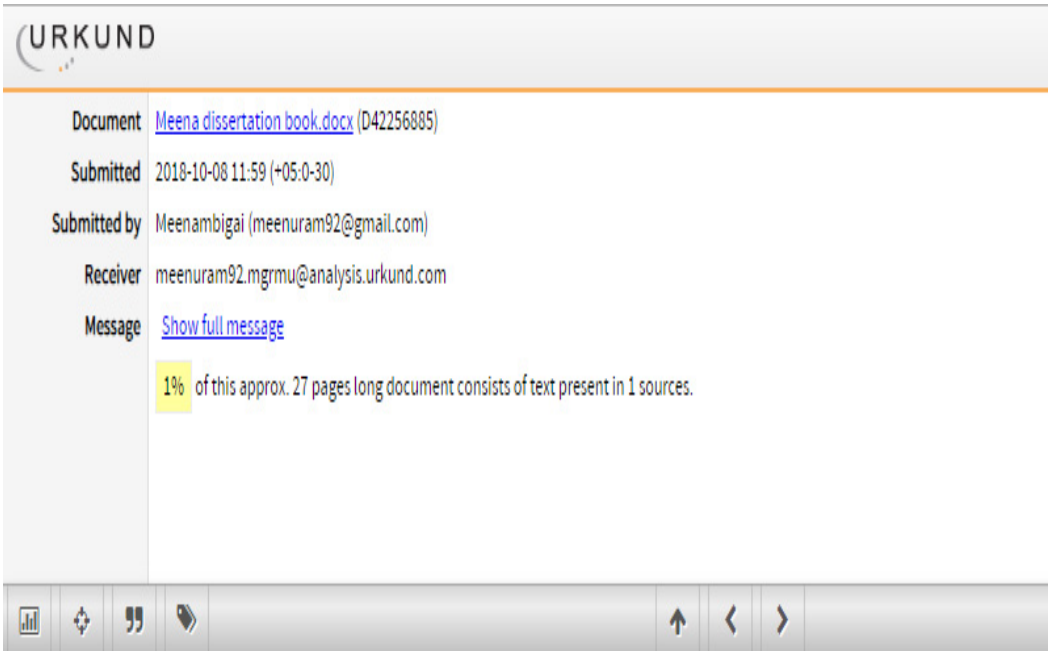
Prof Dr V Nagaraajan
M.D., MNAMS, D.M., Dsc.,(Neuro), Dsc (Hon)
CHAIRMAN
IEC - Madurai Medical College
Madurai

DEAN
Madurai Medical College
Madurai-20

16 APR 2018

# ANNEXURE – X

# ANTIPLAGIARISM CERTIFICATE



# CERTIFICATE

This is to certify that this dissertation titled of "**HISTOPATHOLOGICAL ANALYSIS AND EXPRESSION OF p16 IN SQUAMOUS CELL CARCINOMA OF UPPER-AERODIGESTIVE TRACT"** the candidate **Dr.MEENAMBIGAI** with registration number 201613101 for the award of **M.D** degree in the branch of **PATHOLOGY**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file containing from introduction to conclusion pages and result shows **1** percentage of plagiarism in the dissertation.

**Dr. M. SIVAKAMI, M.D.,**
Professor of pathology,
Department of Pathology,
Madurai Medical College,
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